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Study of Urinary Podocalyxin As an Early Biomarker in Diabetic Nephropathy

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ABSTRACT

Background: Diabetic nephropathy is one of the major complications of diabetes and the leading cause of end-stage renal disease.

Objectives: The main aim of the study was evaluation of urinary podocalyxin as a non-invasive marker for early detection of diabetic nephropathy, through assessing the level of urinary podocalyxin in patients with diabetic nephropathy and assessment of the correlation between urinary podocalyxin levels and severity & grades of diabetic nephropathy.

Patient and methods: A prospective cohort study that was conducted in Internal Medicine Department in Zefta General Hospital and Zagazig University Hospital on 42 cases of diabetic nephropathy. Urinary podocalyxin was estimated by using commercially available podocalyxin ELISA test according to the manufacturer's instructions.

Results: The study showed that the area under the curve for validity of podocalyxin was measured to be 0.996. Also, there was significant association and agreement with sensitivity 97.6% and specificity 100%.

Conclusion: Podocalyxin is a sensitive and valid marker in diabetic nephropathy that can be used for early detection of diabetic nephropathy in its early stages and can predict the prognosis of nephropathy.

Keywords: Diabetic Nephropathy, Prospective Cohort, Podocalyxin.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes is a complex chronic disease that requiring continuous medical care with multifactorial risk-reduction strategies about glycemic control. For patients with DM, self-management education and care are critical to prevent acute complications and long-term complications [(1)]. Diabetic nephropathy is one of the major complications of diabetes and the leading cause of end-stage renal disease [(2)]. It is clinically characterised by proteinuria and progressive renal insufficiency [(3)].

Human Podocytes (glomerular visceral epithelial cells) are highly specialized and terminally differentiated cells, which cover the outline of glomerular basement membrane (GBM) to contribute the final size and charge barrier of glomerular filter to completely limit the leakage of proteins into urinary space [(4)].

Also, it has been demonstrated to be functionally and structurally injured in the natural history of diabetic nephropathy [(5)]. Injuries to podocytes are accompanied by characteristic morphologic changes, as observed in the electron microscopy, including vacuolization, loss of slit diaphragms, effacement of foot process, and detachment from GBM into urine space which is the most severe lesion of podocytes [(6)].

As the result of the proximity of the apical region of Pods to the urinary space, pathological events occurring in this region are expected to be more easily detectable in urine than those occurring in the basal or slit diaphragm regions of Pods [(7)]. The number of podocytes per glomerulus is reduced in diabetic patients with microalbuminuria and/or macroalbuminuria. Moreover, urinary podocytes have been detected in those two stages of diabetes patients [(8)]. Recent studies demonstrated that podocytes detachment presented in diabetic patients with normoalbuminuria by electron microscopic morphometric analysis based on kidney biopsy [(9)].

These findings suggest that detached podocytes and their fragments (marked podocalyxin) might appear in urine of diabetic patients with normoalbuminuria, and that podocalyxin-positive element (PCX + EL) might be a possible marker of early stage of nephropathy, but the role of it remained obscure. Several markers of podocyte injury are available and include nephrin, sinaptopodin, podocalyxin and podocin [(10)]. Podocalyxin (PCX) is the major surface antigen of human podocytes and the expression of PCX on podocytes remains unchanged in various kinds of glomerular nephritis [(11)].

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AIM OF THE WORK

The main aim of the study was evaluation of urinary podocalyxin as a non-invasive marker for early detection of diabetic nephropathy, through assessing the level of urinary podocalyxin in patients with diabetic nephropathy and assessment of the correlation between urinary podocalyxin levels and severity & grades of diabetic nephropathy.

PATIENTS AND METHODS

The study was cross sectional study that was conducted in Internal Medicine department in Zefta General Hospital and Zagazig University Hospital. The study was carried out on patients admitted in Internal medicine department in Zefta General Hospital and Zagazig University Hospital.

Group I: 42 patients (21 mal e +21 female) their ages ranged from 20-70 years. A known clinically and biochemically diabetic patients with diabetic nephropathy in different nephropathy stages (normo, micro and macroalbuminuria) from Zagazig University Hospital (from 1/6/2018 till 1/12/2018).

Group II: 42 apparently healthy volunteers that were included as a control group (21 male + 21 female). Their ages ranged from 20-70 years.

Inclusion criteria: Age: 20 -70 years. Sex: males and females. Known clinically and biochemically diabetic patients with diabetic nephropathy.

Exclusion criteria for cases: Age: < 20 or > 70 years and other causes of nephropathy as: hypertensive nephropathy, autoimmune nephropathy and obstructive nephropathy and end stage renal disease.

All patients included in the study were subjected to the following:

Patients: Patients with type 2 diabetes were divided into 3 groups:

- Group 1: 14 diabetic patients with normo-albuminuria.
- Group 2: 14 diabetic patients with micro-albuminuria.
- Group 3:14 diabetic patients with macro-albuminuria.
- Control group: included 42 apparently healthy volunteers.

Methods: All patients were subjected to**:**

A. Full history taking.

B. Full clinical evaluation.

C. Laboratory investigations:

All patients and controls were subjected to the following:

1) Serum creatinine, blood urea, serum albumin and HbA1C.

2) Complete urine analysis for:

a. Urinary casts.

b. Hematuria.

c. Pyuria.

d. Albumin / creatinine ratio.

3) Urinary podocalyxin was estimated by using commercially available podocalyxin ELISA test according to the manufacturer's instructions.

Ethical approval and written informed consent:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical Analysis

The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows, SPSS Inc. Chicago, IL, USA). One way analysis of variance (ANOVA) was used to compare the results of all examined groups followed by LSD test to compare statistical differences between groups. P value ≤ 0.05 was considered statistically significant.

RESULTS

In the present study, cases showed significantly higher results regarding all measured parameters except for albumin and GFR that were significantly lower (Table 1). In our study ROC Curve for detection of DM cutoff regard studied parameters found that there was significant area under curve with cutoff > 0.5 (Table 2). Regarding association and agreement between cutoff and cases, we found that there was significant association and agreement with sensitivity 97.6% and specificity 100% (Table 3). Comparison among cases with nephropathy and without found that cases with nephropathy had significantly higher all parameters except for albumin that was significantly lower (Table 4). ROC curve for nephropathy cutoff showed significant area under curve with cutoff > 6.2 (Table 5). Comparison among cases regarding state of albuminuria showed that macroalbuminuria cases had significantly higher all parameters except for serum albumin and GFR that were significantly lower. Also, cases with microalbuminuria had significantly higher all parameters except for serum albumin and GFR that were significantly lower (Table 6). Also our study found that urinary podocalyxin had strong significant positive correlation with all parameters except for serum albumin and GFR which showed strong significant negative correlation (figure 1).

Table (1): Lab parameters distribution between studied groups**.**

	Case	Control	t/ Mann Whitney	P
A C ratio	160.5 ± 9.25	14.92 ± 3.51	6.331	0.001**
S creatinine (mg/dL)	2.83 ± 0.5	0.77 ± 0.19	11.357	0.001**
Bl urea (mg/dL)	90.21 ± 2.5	29.4 ± 3.1	19.000	0.001**
S Alb (g/dL)	3.07 ± 0.71	4.55 ± 0.38	-11.838	0.001**
HbA1C	8.64 ± 1.1	3.86 ± 0.51	25.431	0.001**
Urinary podocalyxin	7.17 ± 1.31	0.28 ± 0.09	12.095	0.001**
Urinary albumin	173.28 ± 13.02	16.16 ± 1.16	19.000	0.001**
GFR (mL/min)	71.97 ± 8.18	95.5 ± 0.54	-3.13	0.003 (S)

Table (2): ROC curve for detection of DM cutoff regarding studied parameters**.**

Area	Cutoff	P	95% Confidence Interval	
			Lower Bound	Upper Bound
0.996	> 0.5	0.00**	.987	1.000

Table (3): Association and agreement between cutoff and cases**.**

			Group3		Total	X^2	P	Kappa agreement
			Control	Case				
Cutoff	<0.5	N	42	1	43	80.09	0.001**	0.97
		%	100.0%	2.4%	51.2%			
	>0.5	N	0	41	41			
		%	0.0%	97.6%	48.8%			
Total		N	42	42	84			
		%	100.0%	100.0%	100.0%			

Table (4): Comparison among cases with nephropathy and without (no)**.**

	Nephropathy	No	t	P
A C ratio	218.86 ± 7.9	14.58 ± 3.05	5.092	0.001**
S creatinine (mg/dL)	3.44 ± 0.71	1.29 ± 0.13	10.322	0.001**
Bl urea (mg/dL)	100.1 ± 14.01	65.5 ± 10.7	7.669	0.001**
S Alb (g/dL)	2.67 ± 0.36	4.06 ± 0.21	-12.348	0.001**
HbA1C	9.18 ± 0.78	7.27 ± 0.23	8.253	0.001**
Urinary podocalyxin	9.12 ± 2.0	2.31 ± 0.72	9.891	0.001**

Table (5): ROC curve for nephropathy cutoff**.**

Area	Cutoff	P	95% Confidence Interval	
			Lower Bound	Upper Bound
0.990	> 6.2	0.001**	.968	1.000

Table (6): Comparison among cases regarding state of albuminuria**.**

	Norm albuminuria	Micro albuminuria	Macro albuminuria	F	P
A C ratio	14.58 ± 3.05	53.41 ± 12.48	329.16 ± 12.18	4064.841	0.001**
S creatinine (mg/dL)	1.29 ± 0.13	2.7 ± 0.47	3.94 ± 0.25	261.926	0.001**
Bl urea (mg/dL)	65.5 ± 10.7	87.8 ± 8.7	108.27 ± 10.4	65.179	0.001**
S Alb (g/dL)	4.06 ± 0.21	3.03 ± 0.16	2.43 ± 0.24	211.484	0.001**
HbA1C	7.27 ± 0.23	8.55 ± 0.39	9.61 ± 0.68	74.721	0.001**
Urinary podocalyxin	2.31 ± 0.45	7.12 ± 1.04	7.17 ± 2.7	112.446	0.001**
Urinary albumin	18.53 ± 3.02	69.58 ± 7.76	345.55 ± 19.54	1913.36	0.001**
GFR (mL/min)	90.75 ± 0.86	83.58 ± 3.36	51.72 ± 3.39	784.69	0.001**

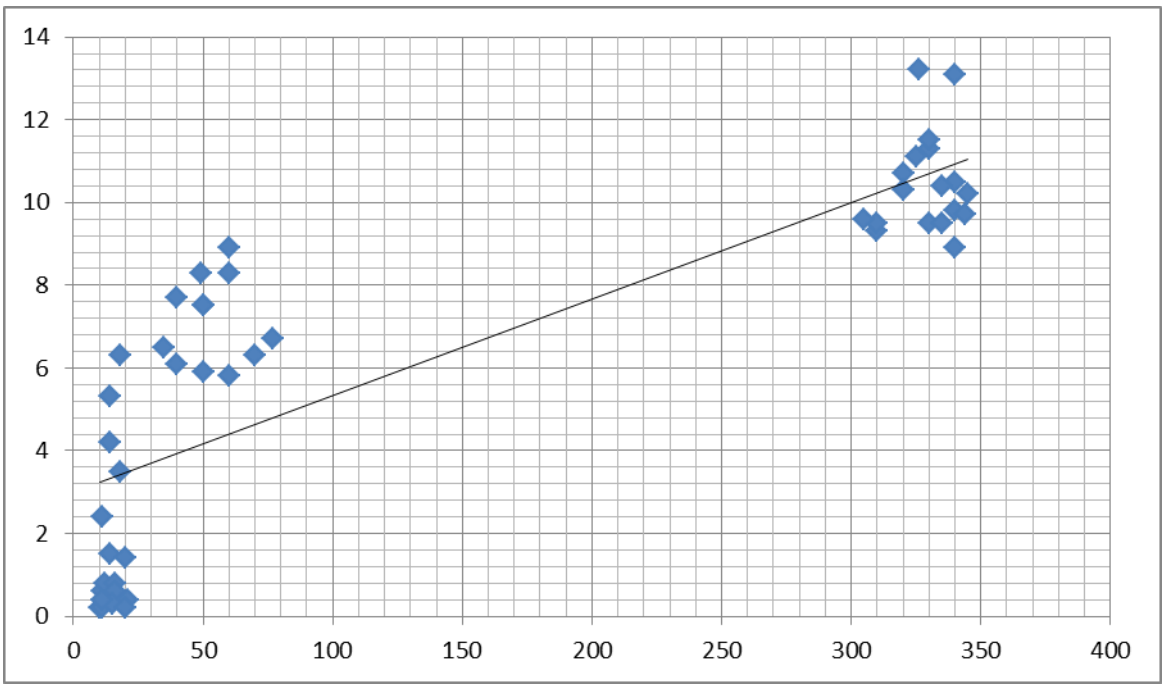

Figure (1): The correlation between podocalyxin and A/C ratio.

DISCUSSION

Diabetes is a rapidly increasing health care problem all over the world, showing a fivefold increase in the prevalence figures during the past 15 years. In parallel, one of the main diabetic complications, nephropathy, is also increasing. Diabetic nephropathy is a devastating chronic event that is characterized by persistent proteinuria, elevated arterial blood pressure, and decline in renal function. Overt nephropathy is diagnosed when the albumin excretion rate (AER) persistently exceeds 300 mg in a 24-h urine collection [(12)].

The decline in renal function and glomerular filtration rate arises from damage to the glomerular filtration barrier. This is accompanied by deterioration of tubular reabsorption, increase of circulating creatinine, and morphologically by accumulation of extracellular matrix and thickening of the basement membrane within the glomeruli. Ultimately, diabetic nephropathy leads to excessive scarring and a nonfunctional end-stage kidney requiring dialysis and transplantation therapies. Microalbuminuria, which is the earliest detectable marker of nephropathy, precedes overt nephropathy and is defined as a persistent AER ranging from 30 to 300 mg/24 h. It is also a strong predictor of subsequent nephropathy in type 1 diabetes [(13)]. At the time of microalbuminuria, the glomeruli already demonstrate advanced glomerulopathy [(14)].

Our study demonstrated that there was non-significant difference between the studied groups as regards demographic data including mean age (46.83 ± 5.28 and 48.54 ± 5.34 years respectively), sex as percent of males and females in control group (52.4% and 47.6% respectively) and in cases group (50% and 50% respectively). This is matching with the study of **Ye *et al.*** [(15)] who conducted their study in Shandong Provincial Hospital, Shandong University in the period from January 2012 to October 2012 on a total of 68 type 2 DN patients and twenty-eight healthy volunteers. They found that there was no significant difference between healthy control and diabetic nephropathy regarding age and sex.

The current study demonstrated that cases were significantly higher regarding all parameters except for albumin that was significantly lower. This comes consistent with what was demonstrated by **Ye *et al.*** [(15)] who observed that the numbers of urinary PCX + EL were significantly increased in DN patients compared with healthy controls. Interestingly, the levels of urinary PCX + EL were significantly increased in DN patients with normoalbuminuria compared with the health controls, suggesting that podocytes injury occurred before the appearance of microalbuminuria in diabetes patients. Thus, urinary PCX + EL might occur in early course of DN and is correlated with a clinical diagnosis of DN, especially in the stage of normoalbuminuria. **Dalla Vestra *et al.*** [(16)] demonstrated that changes of podocytes structure and decrease of podocytes density existed in DN patients with normoalbuminuria by morphometric analysis based on kidney biopsy. **Hara *et al.*** [(17)], who was the first to find that urinary PCX elevated in the DN patients with normoalbuminuria by ELISA. **Hara *et al.*** [(17)], illustrated that regarding

the significant levels of u-PCX, high levels of u-PCX can be considered to reflect marked microvillus transformation and vesicular shedding in glomerular diseases compared to the normal controls. A small amount of vesicles might have been shed by microvilli into the urine of the normal controls by normal physiological turnover, as microvillus transformation is found occasionally on the normal glomerulus.

In our study, the area under the curve for validity of podocalyxin was measured to be 0.996. Also, there was significant association and agreement with sensitivity 97.6% and specificity 100%. This is in agreement with what stated by **Ye *et al.*** [15] who illustrated that the area under the curve for validity of podocalyxin was measured to be 0.996, which demonstrate that the urinary PCX + EL has the highest diagnostic performance. **Zheng *et al.*** [8] reported that the AUC was 0.753 (95% confidence interval, 0.623 to 0.883) for podocalyxin mRNA levels, which demonstrated the highest diagnostic value. The log-transformed threshold providing optimal sensitivity and specificity for podocalyxin mRNA was 23.24. Using the cutoff value of 23.24 derived from the data, podocalyxin mRNA levels predicted DN with a sensitivity of 81.4% and a specificity of 62.5% (p=0.006).

Our study showed that macroalbuminuria was significantly higher regarding all parameters than microalbuminuria**.** This is in harmony with **Żyłka *et al.*** [18] who illustrated that macroalbuminuria was significantly higher regarding all parameters than microalbuminuria**.** This also is in agreement with what stated by **Shoji *et al.*** [7] who evaluated whether urinary podocalyxin levels were associated with the urinary albumin-to-creatinine ratio (ACR). They found that these levels were significantly associated with ACR. When they divided the participants according to the levels of normoalbuminuria, microalbumiuria, or macroalbuminuria, urinary podocalyxin levels were significantly increased in patients with microalbuminuria than in those with normoalbuminuria. Urinary podocalyxin levels in patients with macroalbuminuria were also higher than those in patients with normoalbuminuria, but were not higher than those in patients with microalbuminuria. This may be due to the small number of patients with macroalbuminuria.

Against us was **Nakamura *et al.*** [19] who demonstrated that urinary podocytes absented in the DN patients with normoalbuminuria, but detected in 53% microalbuminuria and in 80% macroalbuminuria patients by immunofluorescence. To explain the conflicted result, some differences between studies should be kept in mind. Some studies detected urinary PCX + EL including the whole podocytes and also podocyte cell debris, which can better reflect the entire podocytes injury in glomerulus, while in other studies only the whole podocytes in the urine were detected. In our study the area under the curve for validity of albumin was measured to be 0.998.

Our study also demonstrated that significant association and agreement sensitivity 94.4% specificity 100.0%. Our study showed that Urinary podocalyxin significantly positively correlated with all parameters including A/C ratio, creatinine, blood urea, HbA1c except with albumin significantly negatively correlated.

Hara *et al.* [17] observed correlation between u-PCX and laboratory data in patients with diabetes. Positive correlations were found between u-PCX level and HbA1c (rs0−0.25, p<0.05), urinary albumin (rs00.35, p<0.01). They explained that this indicate that a hyperglycaemic state causes glomerular capillary-barrier damage, as demonstrated by the increased excretion of u-PCX and albuminuria. **Zheng *et al.*** [8] observed a significant correlation between podocalyxin mRNA and between parameters of renal function including urinary albumin and A/C ratio.

Lioudaki, *et al.* [20] who conducted a study at the Diabetes Clinic of the University Hospital of Heraklion, Crete. They studied 80 patients with type 2 DM and stated that both serum creatinine and urea levels were significantly correlated with DM (r = +0.315, p = 0.008 and r = +0.235, p = 0.05, respectively). This is against what stated by **Shoji *et al.*** [7] who reported that there was strong significant correlation between podocalyxin and albumin. Also, they stated no correlation between podocalyxin and HbA1c.

CONCLUSION

Our results suggest that podocalyxin is a sensitive and valid marker in diabetic nephropathy that can be used for early detection of diabetic nephropathy in its early stages and can predict the prognosis of nephropathy.

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