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Association of Urinary Podocalyxin with Albumin Creatinine Ratio in T2DM Patients

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Abstract

Podocalyxin is a negatively charged protein located in the kidney podocyte membrane, which is a glycosylated cell surface sialomucin of the CD34 family. It is the main component of the glomerular basement membrane charge barrier and has an important role in the regulation of glomerular filtration barrier permeability. There is an argument on the association of PCX with diabetic renal complications and its probability to be a new marker for early diagnosis of diabetic renal complications. The aim of the study was determination of urinary podocalyxin levels in T2DM patients and to analyze its correlation with albumin to creatinine ratio and glycemic control. By consecutive sampling, 82 samples were selected and divided into two groups. The control group consisted of 20 healthy subjects, and the case group was 62 subjects with T2DM. Albumin creatinine ratio, serum creatinine, glycemic control, body mass index and urinary podocalyxin were measured for both groups. Significant higher levels of urinary podocalyxin were found in the case group compared to the control group (p =0.005). There was a significant difference in urinary podocalyxin levels between case groups (normoalbuminuria, microalbuminuria, and macroalbuminuria) (p<0.001). Urinary podocalyxin levels had a significant strong positive correlation with albumin creatinine ratio (p<0.001), and weak positive correlation with serum creatinine and HbA1c. Due to significant differences in urinary podocalyxin levels between the case and control group and within case groups, as well as its positive correlation with albumin creatinine ratio, it might be a good marker for the diagnosis of diabetic kidney disease.

***Keywords*:** *Podocalyxin, ACR, DKD, T2DM*

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I. INTRODUCTION

The disease which is considered one of the most common diseases in the world is Type 2 diabetes mellitus (T2DM). It is caused by two factors: a defect in the secretion of insulin from β-cells or there is no response to insulin by insulin-sensitive tissues (Roden and Shulman, 2019). T2DM causes many complications for patients, the most common one is diabetic kidney disease (DKD), which affects 40% of T2DM patients and leads to end-stage renal disease (ESRD).

Clinically, ESRD is diagnosed by elevated urinary albumin excretion or/and the presence of impaired renal function (Barutta *et al.*, 2021). Even though DKD is usually diagnosed by two common markers, albuminuria and estimated glomerular filtration rate (eGFR) (Cole and Florez, 2020). There is a new marker that researchers have recently discovered called urinary podocalyxin (UPCX) (Ghorab *et al.*, 2020; Xie *et al.*, 2021).

Podocalyxin (PCX) is a negatively charged protein located in the kidney podocyte membrane, which is a glycosylated cell surface sialomucin of the CD34 family (Le *et al.,* 2021). It is the main component of the glomerular basement membrane charge barrier and has an important role in the regulation of glomerular filtration barrier permeability (Akankwasa *et al.*, 2018). PCX is normally secreted by other cells of the body. The cells are mesothelium, vascular endothelial cells, hematopoietic progenitors, and a subset of neurons. It also has a subtle role in tissue development and remodelling outside the kidney (Le *et al.,* 2021).

It has been considered that one of the most important factors in diabetic nephropathy is podocyte injury (Azhari *et al.,* 2023). In the natural history of diabetic nephropathy and

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macrovascular complications, podocytes have been shown to be structurally and functionally affected (Ye *et al.,* 2014).

There is an argument on the association of PCX with DKD and its probability to be a new marker (regardless of the type of sample, urine or serum) for early diagnosis of diabetic renal complications (Ghorab *et al.,* 2020; Khalid and Ali, 2022). Therefore, this study found it interesting to measure UPCX levels in patients with T2DM and analyse its correlation with ACR and glycemic control.

II. Materials And Methods

A. Subjects and study design

This cross-sectional study was performed in Iraq/Duhok City at the diabetic center in Azadi Teaching Hospital and Golan Hospital. By consecutive sampling, 82 samples were selected from October 2021 to April 2022. From these samples, 20 samples were healthy subjects (control group) and 62 samples were patients with T2DM (case group) aged 35-60 years. The exclusion criteria of selecting case group were chronic diseases (hypertension, thyroid diseases, and cardiovascular diseases), renal diseases, malignant diseases as well as pregnancy.

The following information; (name, age, gender, phone number, duration of disease, blood pressure, height, weight, and waist circumference), listed in a questionnaire, was collected from each patient who accepted to participate and fulfilled the research criteria.

After verbally verifying that patients were fasting, a blood sample of 4 mL was drawn from the arm of them. Two mL of blood was collected into the EDTA tube for measuring HbA1c and the remaining were collected into the gel tube to be centrifuged at 3000 rpm for 10 minutes to separate serum for estimation of FBS and creatinine. A fresh urine sample was collected from each patient into a plain tube for measuring albumin, creatinine, and PCX levels.

Case group were divided into three groups according to albumin creatinine ratio (ACR); 34 patients had normoalbuminuria (ACR< 30 mg/g creatinine), 20 patients had microalbuminuria (ACR 30-300 mg/g creatinine), and 8 patients had macroalbuminuria (ACR >300 mg/g creatinine). Healthy people were asked randomly to volunteer in this research as the control group. People who accepted to participate were proceeded with the same data and sample collection process as the case group followed.

B. Measurements

The concentration of PCX in urine was determined by the Human PCX ELISA Kit (Catalog No: E-EL-H2360, Sensitivity: 0.10 ng/mL, Elabscience Biotechnology Inc. USA). Urine albumin, serum creatinine (SCr), urine creatinine, FBS, and HbA1c were analyzed by the biochemistry autoanalyzer Cobas series 6000.

ACR was calculated by dividing the concentration of albumin in urine by the concentration of creatinine in urine. Cockcroft-Gault equation was used to calculate eGFR {((140–age) x weight in kg)/ (72 x SCr in mg/dL)(x 0.85 if female)}. Anthropometric assessments, including body mass index (BMI) and waist circumference (WC), were also measured.

C. Statistical analysis

The study used SPSS version 20 to analyze all data. T-test and one-way ANOVA were applied for comparison between groups. In addition, the association between UPCX and other parameters was estimated by Pearson's correlation coefficient. The level of statistical significance (p-value) was set at <0.05.

D. Ethical considerations

The study procedure was consulted and accepted by the Ethical Committee of the General Directorate of Health in Duhok (18082021-8-24).

III. Results

The comparison of clinical data and laboratory parameters among the study groups is summarized in Table 1. There was a significant difference between the case and control group regarding age p=0.020, BMI p<0.001, FBS p<0.001, HbA1c p<0.001, SCr p<0.001, eGFR p=0.046, ACR p=0.015, and UPCX p=0.006, which were higher in the case group.

The comparison between case groups is summarized in Table 2 and showed a significant difference in SCr p= 0.001, HbA1c p=0.034, ACR p< 0.001, UPCX p< 0.001, which had the highest levels in macroalbuminuria, and eGFR p=0.006 had the lowest levels.

Table 1. Comparison of clinical data and laboratory parameters among case and control group

Variables	Control Group (n=20) Mean ±SD	Case Group (n=62) Mean ±SD	P value
Gender (male/female)	14/6	25/37	
Age	43.10 ±9.62	48.4 ±8.45	0.020
BMI (kg/m2)	25.59 ±3.39	30.92 ±4.82	<0.001
WC (cm)	92.10 ±7.98	100.81 ±10.31	0.001
FBS (mg/dL)	85.80 ±13.49	191.98 ±71.00	<0.001
HbA1c %	5.36 ±0.26	8.88 ±2.03	<0.001
SCr (mg/dL)	0.57 ±0.14	0.78 ±0.22	<0.001
eGFR (mL/min)	124.61 ±28.64	143.49 ±38.29	0.046
ACR (mg/g creatinine)	6.18±2.80	87.23±145.08	0.015
UPCX (ng/mL)	4.30 ±2.40	11.61 ±11.49	0.006

The correlation of UPCX with clinical data and laboratory parameters in the case group is showed in Table 3. UPCX revealed a strong positive correlation with ACR p<0.001, and a weak positive correlation with HbA1c p=0.034, SCr p=0.007, as shown in Figure 1, 2, and 3 respectively. The correlation between UPCX and eGFR was statistically not significant p=0.108 (Figure 4). There was no significant correlation between UPCX and BMI, FBS and duration of diabetes.

Table 2. Comparison of clinical and laboratory data between case groups

Variables	Normo n=34 Mean ±SD	Micro n=20 Mean ±SD	Macro n=8 Mean ±SD	P value
Gender (male/female)	13/21	6/14	3/5	
Age	48.68 ±8.31	46.8 ±9.71	51.63±4.56	0.390
BMI (kg/m2)	31.18±4.12	31.09 ±6.50	29.36 ±2.13	0.626
WC (cm)	100.38 ±9.33	100.15 ±12.20	104.25 ±9.76	0.605
FBS (mg/dL)	186.47±76.74	200.45±75.85	194.25±13.92	0.785
HbA1c %	8.40±1.65	9.09 ±1.89	10.41±3.05	0.034
SCr (mg/dL)	0.60±0.16	0.57±0.15	0.86 ±0.27	0.001
eGFR (mL/min)	147.60±36.15	151.02±40.78	107.19±18.80	0.013
ACR (mg/g creatinine)	13.96±7.84	68.86 ±38.04	444.55±79.14	<0.001
UPCX (ng/mL)	5.49 ±5.39	15.69±12.48	27.48 ±8.6	<0.001

Table 3. Correlation between UPCX and other laboratory findings in the case group

Variables	r	P-value
BMI (kg/m^2)	0.025	0.847
Duration of diabetes (years)	-0.035	0.788
FBS (mg/dL)	0.080	0.539
HbA1c %	0.269	0.034*
SCr (mg/dL)	0.339	0.007*
eGFR (mL/min)	-0.206	0.108
ACR (mg/g)	0.610	<0.001*

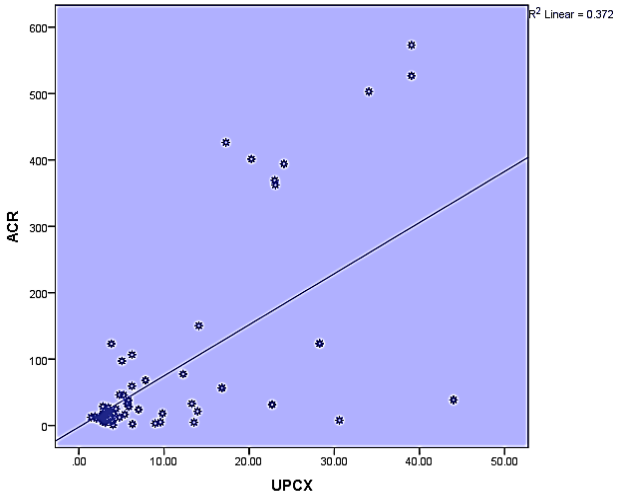

Figure 1. Scatter plot shows a strong positive correlation between UPCX and ACR.

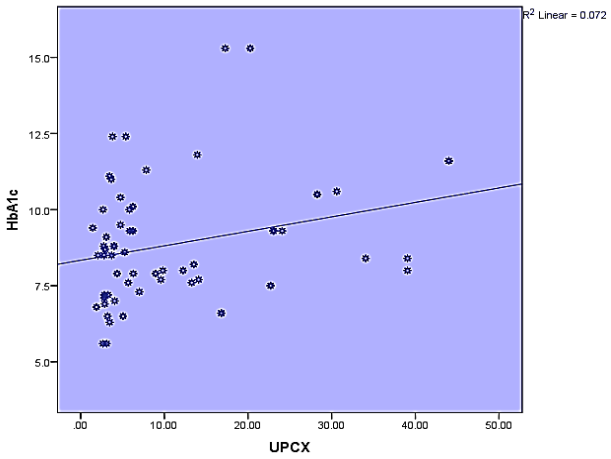

Figure 2. Scatter plot shows a weak positive correlation between UPCX and HbA1c

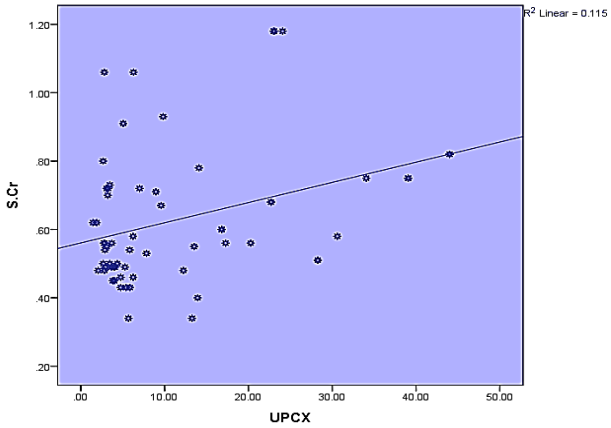

Figure 3. Scatter plot shows a weak positive correlation between UPCX and SCr

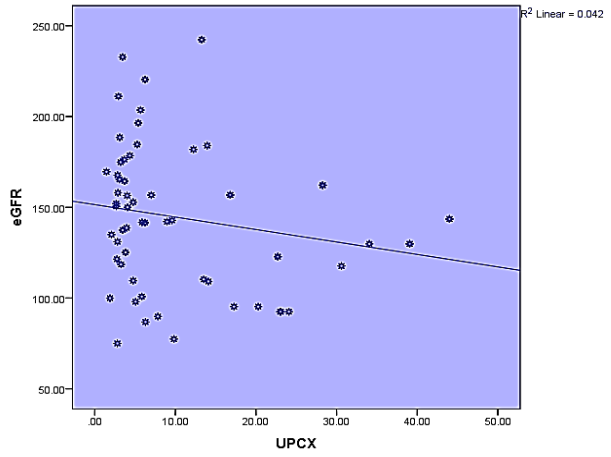

Figure 4. Scatter plot shows a non-significant correlation between UPCX and eGFR.

IV. DISCUSSION

The DKD diagnosis depends on laboratory findings (declined GFR, albuminuria). For diabetic patients to be diagnosed with DKD, their eGFR is reducing below 60 mL/min/1.73 m2 persistently and/or ACR must be more than 30 mg/g in two measurements with at least a 3-month difference (Alicic *et al.,* 2017). Endothelial cells, mesangial cells, and podocytes are three cell types found in the glomerulus, which play a crucial role in DKD development (Betsholtz *et al.,* 2007). However, it is difficult to determine which cell types are most impacted during DKD development without a renal biopsy. It is inappropriate to undertake renal biopsies in all patients with diabetes, particularly in the early stages of DKD, as they are an invasive test (Shoji *et al*., 2016).

There is many confounding issues associated with albumin in the urine that cause a reduction of its sensitivity and specificity in the diagnosis of the early stages of DKD, which are acute illness, cardiac failure, urinary tract infection, and exercise (Li *et al.,* 2017). Additionally, not all T2DM patients with microalbuminuria will end up with ESRD, and 30% of them may have normoalbuminuria. According to previous research, microalbuminuria is present once a significant renal injury has occurred (Lee and Choi, 2015; Chida *et al.,* 2016), and a decline in eGFR is a final outcome of kidney disease (Betsholtz *et al.,* 2007). Because of these limitations, there is an urgent need to find more sensitive and specific biomarkers for the identification or early detection of DKD. Due to DKD being considered podocytopathy, specific podocyte protein measurement, such as PCX, could determine an injury to the podocyte.

Podocalyxin was measured in serum (SPCX) in a study conducted by Khalid and Ali (2022) and its correlation with diabetes and DKD biomarkers was analyzed in order to show the probability of using it as a biomarker for DKD instead of UPCX. The researchers did not find any significant correlation between SPCX and DKD biomarkers and they concluded that it cannot be used as a biomarker for DKD. Therefore, the current study decided to repeat the same study by using a urine sample instead of a serum sample, as in previous studies (Xie *et al*., 2021), to measure the PCX level. The case group in the present study had significantly higher UPCX levels than the control group. The same findings were showed by (Kostovska *et al.*, 2020; Shelbaya *et al.*, 2020) who found that UPCX had a higher level in T2DM patients. Additionally, we found a significant difference in UPCX levels among case groups; the macroalbuminuria group had the highest levels. The same findings were seen in research conducted in 2014 and 2020 (Ye *et al.*, 2014; Ghorab *et al.*, 2020). As in previous studies (Kostovska *et al.,* 2020; Shelbaya *et al.,* 2020; Ghorab *et al.,* 2020; El-ballat *et al.,* 2023), our results confirmed the significant strong positive correlation between UPCX and ACR.

Besides apoptosis, detachment from GBM, cellular hypertrophy, and foot-process effacement that occur in podocytopathy, the transformation of microvilli appears on the surface of apical cells, which are located on the damaged podocytes (podocytes exist outside the GBM). Pathological conditions that happen in the apical region of podocytes are predicted to appear more in urine than those happening in the slit diaphragm or basal regions of injured podocytes (Hara *et al.*, 2005) because this region is very near to the urinary space. Hara *et al.,* (2012) established that the apical surface of cells sheds vesicles into the urine as a result of microvilli tip vesiculations and then UPCX reflects this microvillus shedding. They applied immunofluorescence and immunoelectron microscopy using an anti-PCX antibody to demonstrate the existence of vesicles in the urine.

Due to the strong positive correlation of UPCX with ACR, it might be a good marker for DKD diagnosis. However, further research with a larger sample size is required to support our result and to ensure the correlation of UPCX with ACR because the limitation of this study is a small sample size.

In the current study, a significant weak positive correlation between SCr and UPCX was found. Similar findings were shown by Kostovska *et al*. (2020) and Shelbaya *et al*. (2020). Although SCr indicates renal impairment at a relatively late stage, renal function is decreased by up to 50% before a rise in SCr is observed (Bouchard, 2021), its correlation with UPCX might indicate the probability of using UPCX as a DKD biomarker.

A significant weak positive correlation between HbA1c and UPCX was shown in this study and it is consistent with the findings of the previous studies (Shelbaya *et al.,* 2020; Kostovska *et al.,* 2020), which may indicate a role of uncontrolled diabetes in glomerular capillary-barrier damage. Studies have demonstrated that patients with microalbuminuria can prevent the progression of DKD by maintaining strict glycemic control. In addition, glycemic control that targets an HbA1c level of <7% is recommended to avoid the progression of DKD (Miyamoto and Shikata, 2021).

Unlike the research conducted by Mohamed *et al.* (2016) who established a negative correlation between UPCX and eGFR, we did not find significant correlation between them. Shoji *et al.* (2016) and Kostovska *et al.* (2020) also confirm the absence of a correlation between the two variables. Other conditions may cause a decline in eGFR levels such as dyslipidemia, glomerular hyperfiltration, and hypertension. Obesity and sex could also contribute to decreasing eGFR (Porrini *et al*., 2015).

Being overweight and obese are very strongly correlated with the prevalence of DKD. Obesity is involved in the aetiopathogenesis of T2DM and the development of its complications (Zhang *et al.,* 2023). However, UPCX in this study showed no correlation with BMI because there were no significant differences in BMI among case groups. Shelbaya *et al.* (2020) also showed no significant correlation between UPCX and BMI.

Moreover, UPCX in this study showed no correlation with FBS and duration of diabetes. Research conducted in 2020 also established no correlation between UPCX and duration of diabetes (Kostovska *et al.,* 2020).

V. Conclusion

According to the outcomes of this study, UPCX might be a good marker for DKD diagnosis due to its strong positive correlation with ACR. The significant differences in UPCX levels between the case and control groups and among case groups increase the probability of being UPCX as a marker for DKD. Its positive correlation with SCr, and HbA1c increases this probability. However, due to the study limitation, which is a small sample size, further research with a larger sample size is required to support our results.

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