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# Assessment the level of Urinary podocalyxin as an early biomarker in Diabetic Nephropathy

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## ABSTRACT

**Background**: For a long time, measuring urinary albumin and glomerular filtration rate (GFR) was considered the key to diagnosing kidney disease. Due to the lack of early diagnosis, the search for new markers that can provide early diagnosis and avoid complications has become increasingly important. Podocyte cell injury or dysfunction plays an important role in the development and progression of many kidney diseases . This enhances the potential of using podocyte cell product targets specially in urine as useful clinical indicators for the diagnosis and monitoring of kidney diseases.

**Methods**: A cross-sectional study will be carried out to a group of 70 diabetic patients with diabetic nephropathy 20 healthy control. A total of 25 mL urine was collected. Alb/Cr ratio in a random urine sample . Serum creatinine, serum albumin and HbA1C. Urinary Albumin / creatinine ratio. Urinary podocalyxin, nephrin and podocin were determined by using commercially available ELISA test.

**Results:** the study revealed that patients with normoalbuminuria have higher urinary PCX. Level than healthy control group as well as revealed that 50% of patients with normoalbuminuria have higher urine podocalyxin levels. These findings suggest that in the early stages of kidney damage urinary podocalyxin first appears in the urine preceding microalbumin. The results obtained showed that urinary podocalyxin level was significantly increased in both UM/CR and CKD staging subgroups compared with the healthy control group. A gradual increase in urinary podocalyxin level with CKD stage, especially in 2 and 3 stages, and the higher sensitivity of urinary podocalyxin as compared to UM/CR ratio in early detection of kidney damage was demonstrated.

**Conclusions:** Diabetes patients had much greater amounts of podocalyxin, nephrin, and podocin in their urine compared to the healthy control group.

***Keywords:*** *diabetes, kidney disease. podocyte. Podocalyxin and podocyte markers*

## 1. INTRODUCTION

Even though diabetic kidney disease (DKD) does not follow an exact timeline, patients with diabetes followed up for 10 years in the UK were noted to progress to microalbuminuria at a rate of 2% per year after the diagnosis of diabetes[(1)] .

For a long time, measuring urinary albumin and glomerular filtration rate (GFR) was considered the key to diagnosing kidney disease. Due to the lack of early diagnosis, the search for new markers that can provide early diagnosis and avoid complications has become increasingly important.

podocyte cells contribute to maintaining the blood-urine barrier, preventing proteins and large molecules from entering the urinary ultrafiltrate. Therefore, podocyte cell injury or dysfunction plays an important role in the development and progression of many kidney diseases[(2)] . This enhances the potential of using podocyte cell product targets specially in urine as useful clinical indicators for the diagnosis and monitoring of kidney diseases. Many previous studies revealed that the urinary level of podocyte - associated molecules may predict the prognosis of DKD one of most important one is the podocalyxin which is the major transmembrane protein expressed on the apical side of podocyte and have a role in glomerular permeability and podocyte morphology regulation [(3,4)] as well as showed that urinary podocalyxin level of DKD patients was higher than that of the control group, and the level positively correlated with urinary albumin excretion, indicating that urinary podocalyxin level might serve as a biomarker of DKD [(5,6)] .

In the present study, we compared the role of urinary podocalyxin and other podocyte markers with GFR and urinary albumin/ creatinine ratio to know which of them is more sensitive and more important in the early diagnosis of the disease.

In addition, most previous studies focused on measuring the level of these markers in a kidney biopsy, which is a complex process and not easy to perform[7,8] . Therefore, in this study, we will measure the level of these markers in urine and compare them with the classic tests that diagnose the disease to demonstrate their efficiency in early and accurate diagnosis of the disease.

## 2. MATERIAL AND METHODS

A cross-sectional study will be carried out to a group of 70 diabetic patients with diabetic nephropathy 50 male and 20 female and 20 healthy control group 15 male and 5 female . All the subjects enrolled in this study were diagnosed under specialist supervision. Serum and urine samples will obtain from all participants.

A total of 25 mL urine was collected and centrifuged at 2000 rpm for 10 min. The supernatant was aliquoted to volume to 0.5 ml. . A mixture of glacial acetic acid, ethanol, and double-distilled water was added up to a total of 1 mL. Samples were stored at -20°C and aliquots were thawed for ELISA assays.

Diabetic patients were divided into four groups according to their stage of kidney disease . There are 5 stages of kidney disease. Determination the stage of kidney disease based on the presence of kidney damage and glomerular filtration rate (GFR) normal , mild, moderately , sever , and kidney failure.

Another division based on the albuminuria in which all patients included in the study were divided into Normoalbuminuria , Microalbuminuria and. Macroalbuminuria . In the present study we prefer to measure the Alb/Cr ratio in a random urine sample . Serum creatinine, serum albumin and HbA1C. Urinary Albumin / creatinine ratio. Urinary podocalyxin, nephrin and podocin were determined by using commercially available ELISA test according to the manufacturer's instructions.

## 3. RESULTS

According to age and sex Our study revealed that there was non-significant difference between the studied groups as regards demographic data including mean age and sex between patients and control groups . The study reveals that the majority of diabetic patients were in older age with long duration of diabetes ( 64.27 ± 10.9) as shown in table (4-1) .

The current study demonstrated that cases were significantly higher regarding the levels of UA/C ratio and HBA1c parameters in diabetic patients than that of healthy group table (4-1). Also the present study confirmed that there was a decline in glomerular filtration rate in patients than that of control group.

**Table (4-1) showed the characteristics of diabetic patients and control groups**

| parameters | Groups | No. | Mean | Std. D. | P Value |
|---|---|---|---|---|---|
| AGE | diabetic patients | 70 | 64.27 | 10.9 | |
| | heathy control | 20 | 61.70 | 13.1 | Non. sig. |
| eGFR | diabetic patients | 70 | 63.1 | 25.2 | |
| | heathy control | 20 | 98.0 | 4.5 | Sig. |
| UA/C ratio | diabetic patients | 70 | 232.6 | 167.3 | . |
| | heathy control | 20 | 20.7 | 5.7 | Sig. |
| FBS | diabetic patients | 70 | 97.5 | 19.3 | |
| | heathy control | 20 | 91.9 | 8.2 | Non sig. |
| HBA1C | diabetic patients | 70 | 7.4 | .74 | |
| | heathy control | 20 | 5.5 | .53 | Sig. |

An important finding of the present study was that the patients group had higher Urinary Podocalyxin (PCX) levels than the control group table (4-2) . In addition, a significant higher Urinary PCX levels were found in patients with macroalbuminuria than in those with normoalbuminuria and microalbuminuria .fig (4-1). However, our results showed a strong positive correlation between urinary PCX level and UACR, fig (4-5

**Table (4-2) showed the levels of podocyte markers in diabetic patients and control group.**

| Parameters | group | No. | Mean | Std. D. | P value |
|---|---|---|---|---|---|
| U. PCX/UCre. ng/umol | diabetic patients | 70 | 36.0 | 13.2 | |
| | heathy control | 20 | 15.1 | 5.1 | Sig. |
| U. Nephrin/UCre. ng/umol/l | diabetic patients | 70 | 16.3 | 5.0 | |
| | heathy control | 20 | 2.4 | .9 | Sig. |
| U. Podocin/Ucre. ng/Umol/l | diabetic patients | 70 | 80.2 | 24.9 | |
| | heathy control | 20 | 40.7 | 17.0 | Sig. |
| Serum.PCX ng/dl | diabetic patients | 70 | 148.6 | 22.1 | |
| | heathy control | 20 | 144.3 | 25.7 | Non sig. |

Our study revealed that patients with normoalbuminuria have higher urinary PCX. Level than healthy control group as well as revealed that 50% of patients with normoalbuminuria have higher urine podocalyxin levels. These findings suggest that in the early stages of kidney damage urinary podocalyxin first appears in the urine preceding microalbumin.

The results obtained showed that urinary podocalyxin level was significantly increased in both UM/CR and CKD staging subgroups compared with the healthy control group. A gradual increase in urinary podocalyxin level with CKD stage, especially in 2 and 3 stages, and the higher sensitivity of urinary podocalyxin as compared to UM/CR ratio in early detection of kidney damage was demonstrated.

**Table (4-3 ) laboratory level of parameters in different groups**

| **parameter** | **Healthy control No. 20** | **Normo Albuminuria No.20** | **Micro Albuminuria N0.30** | **Macro Albuminuria N0.40** | P Value |
|---|---|---|---|---|---|
| Age | 61.9 ± 13.1 | 65.7± 11.1 | 64.5 ± 11.3 | 63.8 ± 10.5 | Non Sig. |
| eGFRml/min. | 98.0 ± 4.5 | 92.6 ± 6.5 | 82.2 ± 13.1 | 37.7 ± 11.0 | Sig . |
| UA/Cre.ratio | 20.7 ± 5.7 | 27.7± 6.3 | 96.0 ± 45.7 | 414.8 ± 61.1 | Sig. |
| HBA1c | 5.5 ± 0.5 | 6.5± 0.5 | 7.5 ± 0.7 | 7.4 ± 0.8 | Sig. |
| FBG | 91.9 ± 19.3 | 91.4 ± 8.2 | 98.6 ± 18.3 | ± 20.8 96.2 | Non sig |
| Pcx/U Cre. ng/umol/l | 15.1 ± 5.1 | 18.4± 6.4 | 26.1 ± 6.1 | 49.1± 7.0 | sig |
| Nephrin/Ucre. ng/umol/l | 2.4 ± 0.9 | 5.6± 1.9 | 13.1 ± 2.8 | 20.5± 4.0 | sig |
| Podocin/Ucre. Ng/umol/l | 40.7 ± 17.0 | 44.6± 15.9 | 72.7 ± 25.3 | 90.2± 20.8 | sig |
| S. podocalxin | 140.3 ± 20.7 | 144.5± 25.8 | 150.3± 22.6 | 146.2± 21.6 | Non sig |

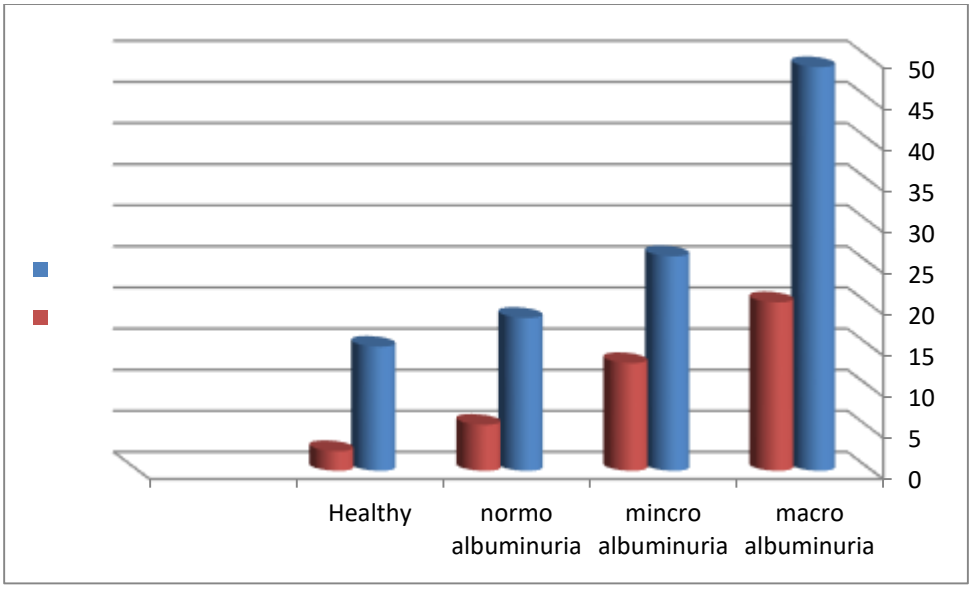


**Figure (4-1) urinary podocalyxin and nephrin levels in subgroups of patients divided according to UM/CR**

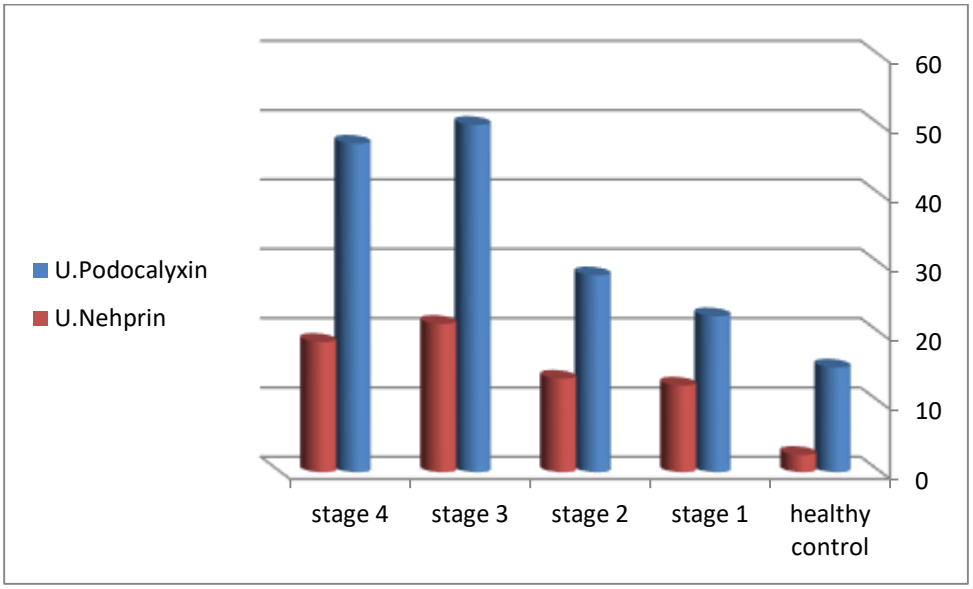


**Figure (4-2) urinary podocalyxin and Nephrin levels in subgroups of patients divided according to CKD stage.**

The current study showed a negative correlation between U.PCX and eGFR in the case and control group while a strog positive correlation was found between U.PCX and albumin/creatnine ratio . As shown in figure (5,6).

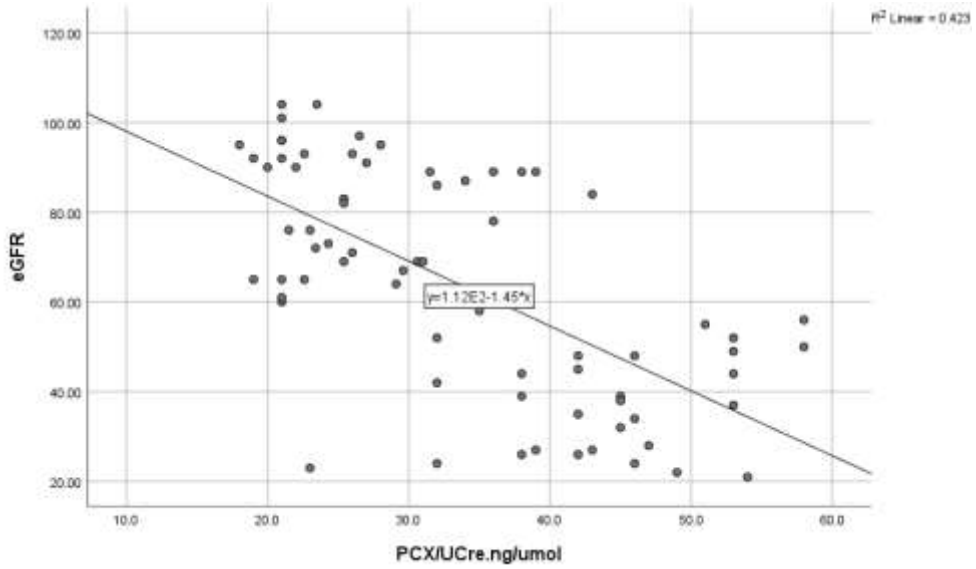


**Figure ( 4-5) show the correlation between U. Podocalyxin and eGFR serum levels in diabetic patients group**

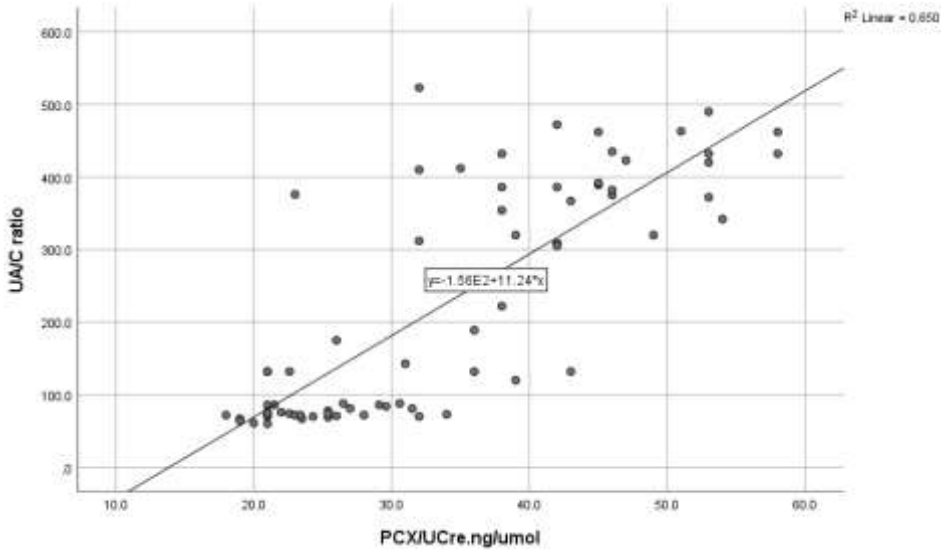


**Figure ( 4-6) show the correlation between U. Podocalyxin and UA/C ratio serum levels in diabetic patients group**

## 4. DISCUSSION

An important finding of the present study was that the patients group had higher Urinary Podocalyxin (PCX) levels than the control group table (4-2) . Similar results were found by (Kostovska et al., 2020; Shelbaya et al., 2020) [10,11] , who reported that urinary podocalyxin (U.PCX) levels were higher in diabetic patients. In addition, a significant higher Urinary PCX levels were found in patients with macroalbuminuria than in those with normoalbuminuria and microalbuminuria .fig (4-1). The high levels of U.PCX in micro and macroalbuminuria patients were confirmed by (Ghorab et al., 2020)[12] due to the origination of U.PCX from the small villi of vesicle in injured podocytes . However, our results showed a strong positive correlation between urinary PCX level and UACR, fig (4-5) in the same manner with previous studies conducted on U.PCX and presented a significant positive correlation between them (Kostovska et al., 2020; Shelbaya et al., 2020; Ghorab et al., 2020) [10,11,12] they were found urinary PCX levels were associated with levels of UACR and also increased in diabetic patients with normal levels of urinary albumin .

PC X, a negatively charged glycoprotein, expressed in the membrane of podocytes, plays an essential role in maintaining the function of glomerular podocytes[13] . Podocalyxin ac t to prevent negatively charged protein from leaking into the urine , maintain the separation of adjacent podocytes and prevent the adhesion between epithelial cells and capillary loops[14,15] .

By the using of immunofluorescence to determine the expression of PCX in in the urine, they found that the increased level of urinary PCX in originated from the apical membrane region of podocytes, rather than shedding fragments from podocytes, indicating that PCX in urine was an earlier indicator than GFR [16,17] .

In glomerular diseases, the level of urinary podocalyxin and the number of urinary podocytes were associated with the proportion of segmental sclerosis. The expression of the kidney function biomarkers serum creatinine, eGFR, and albuminuria was linked with urinary PDX mRNA[18] .

Our study revealed that patients with normoalbuminuria have higher urinary PCX. Level than healthy control this finding suggest that urinary podocalyxin appears earlier than albuminuria in early renal diseaseas shown in table (4-3) and fig (4-1 ).

The present study revealed that 50% of patients with normoalbuminuria have higher urine podocalyxin levels. These findings suggest that in the early stages of kidney damage urinary podocalyxin first appears in the urine preceding microalbumin.

All diabetic patients were divided into subgroups according to urine microalbumin/creatinine ratio and the stage of kidney dammage . The results obtained showed that urinary podocalyxin level was significantly increased in both UM/CR and CKD staging subgroups compared with the healthy control group. A gradual increase in urinary podocalyxin level with CKD stage, especially in 2 and 3 stages, and the higher sensitivity of urinary podocalyxin as compared to UM/CR ratio in early detection of kidney damage was demonstrated. It was concluded that urinary podocalyxin may be an important and highly sensitive marker for early diagnosis of diabetic dammage in patients with diabetic patients. As shown in table ( 4-3 ) and fig (4-1 ).

The current study showed a negative correlation between S.PCX and eGFR in the case and control group. Similar findings were reported by Mohamed et al. [19], who established a negative correlation between U.PCX and eGFR in patients with T2DM. Conversely, other study showed no correlation between the two variables[20]. Serum creatinine and S.PCX were significantly positively correlated in the case group. Similar findings were reported by Kostovska et al. (2020)[21] and Shelbaya et al. [22] , who reported a positive correlation of U.PCX with serum creatinine. 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[20].

## 5. CONCLUSIONS

Diabetes patients had much greater amounts of podocalyxin, nephrin, and podocin in their urine compared to the healthy control group, according to the data. Urinary podocalyxin was shown to be more sensitive than UM/CR ratio in detecting diabetic nephropathy in its early stages, and our work shows that its level gradually increases with chronic kidney disease stage, particularly in stages 2 and 3.

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