Urinary levels of podocalyxin as a marker for podocytopathy in patients with metabolic syndrome having high body mass index: a diagnostic test accuracy study

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Aim
To assess the adequacy of urinary podocalyxin (PCX) as a biomarker for early detection and disease progression of podocytopathy in high-BMI patients with metabolic syndrome with normal and impaired kidney functions.
Patients and methods
This is a cross-sectional study where all participants were subjected to full history taking, complete physical examination with assessment of BMI, and laboratory investigation to assess the inclusion and exclusion criteria. Then, a fresh morning urinary sample was obtained to measure both urinary albumin/creatinine ratio (UACR) and urinary PCX, where urinary PCX is measured by using human PCX ELISA kit.
Results
Urinary PCX showed that it can differentiate between group BI (albuminuric with normal renal functions) and BII (impaired renal functions) at the suggested cutoff point of 285 pg/ml, with 52% sensitivity and 45% specificity with low positive predictive value 48.8% and negative predictive value 48.6% by using receiver operating characteristic analysis, with *P* value 0.008, but it cannot differentiate between group A (completely normal renal functions) and neither of group BI nor BII.
Conclusion
Although urinary PCX is more sensitive for the diagnosis of early diabetic nephropathy than UACR and is used as a marker of chronic kidney disease progression in diabetic patients, UACR is still the gold standard for diagnosis and follow-up of diabetic nephropathy. Urinary PCX is nonspecific and cannot be related to BMI in patients with metabolic syndrome.

Keywords:
metabolic syndrome, podocytopathy, urinary podocalyxin

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Introduction

Increased BMI including overweight and obesity is a major health problem in both developed and developing countries. Currently, it is considered an epidemic disease. It is not only a cosmetic problem but also increases the risk of health problems as well, such as cardiac disease, diabetes, and hypertension, which may cause mortality. The good news is that even the slightest weight reduction can improve health problems [1]. Obesity alone is a major risk factor for chronic kidney disease (CKD) and end-stage renal disease (ESRD). In combination with diabetes, they account for 70% of all ESRD cases [2].

Metabolic syndrome is one of the major consequences of obesity and high BMI, where it reaches up to 50% of the severely obese population. It has other synonyms, which are the deadly quartet, syndrome X, and Reaven's syndrome. Many criteria are implied for the diagnosis, but the most commonly used is National Adult Education Programme Adult Treatment Panel III (NCEP-ATP III). Other organizations also defining it are WHO and the International Diabetes Federation [3,4].

The NCEP-ATP III diagnose metabolic syndrome if the patient has at least three of the following five conditions: fasting glucose equal or above 100 mg/dl or receiving drugs for hyperglycemia, blood pressure equal or above 140/90 mmHg or receiving drugs for hypertension, triglycerides equal or above 150 mg/dl, high-density lipoprotein cholesterol is less than 40 in men and 50 in women, and waist circumference is more than 102 cm in men and 88 cm in women. The modified NCEP-ATP III criteria suggest that

central obesity is ethnicity specific, where in USA, as per NCEP-ATP III, males more than or equal to 102 cm and females more than or equal to 88 cm; in Europoids, males more than or equal to 94 cm and females more than or equal to 80 cm; in South Asians and Chinese, males more than or equal to 90 cm and females more than or equal to 80 cm [5] which has shown to be better than International Diabetes Federation criteria [6].

Both of them affect the kidneys by causing podocytopathy. Podocytopathy is defined as any damage or functional alteration of the podocyte, which is presented as proteinuria [7,8].

The diagnosis of podocytopathy can be done by morphological assessment, immunohistochemistry, circulating biomarkers, urine biomarkers, and mutation analysis [9].

So, the search for a suitable urinary biomarker is imperative as a noninvasive tool for the diagnosis of podocytopathies.

Aim

The study was designed to assess the adequacy of urinary podocalyxin (PCX) as a biomarker for early detection and disease progression of podocytopathy in high-BMI patients with metabolic syndrome with normal and impaired kidney functions.

Patients and methods

This study was conducted on 80 patients divided into two groups:

(1) Group A: 40 patients with BMI equal or above 25 with metabolic syndrome and with normal renal functions and not taking angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs).
(2) Group B: 40 patients with BMI equal or above 25 and with metabolic syndrome and with nephropathy. Further subdivision of group B into group BI and BII was done, where group BI (24 patients) had normal serum creatinine and taking ACEi or ARBs with the previous history of proteinuria or currently proteinuric with urinary albumin/creatinine ratio (UACR) more than 30, and group BII (16 patients) had increased serum creatinine with reduction of glomerular filtration rate (GFR) till stage 4 CKD with or without proteinuria and with or without being on ACEi or ARBs medications.

Inclusion criteria

The following were the inclusion criteria:

(1) Age between 21 and 60 years (to avoid the physiologic age-related changes in GFR).
(2) Patients with metabolic syndrome (type 2 diabetes mellitus, hypertension, dyslipidemia and increased waist circumference >102 cm in men and >88 cm in women) [10] and with high BMI.

Exclusion criteria (any patient has a possible cause of chronic kidney disease or proteinuria)

The following were the inclusion criteria:

(1) Liver disease (either by history or any increase in serum transaminases above reference range).
(2) Cardiac disease (either by the history of dyspnea, chest pain, paroxysmal nocturnal dyspnea and orthopnea or receiving any cardiac medications).
(3) Primary glomerulopathy causing nephritic or nephrotic syndrome (by history, examination, urine analysis, UACR, and serum urea and creatinine).
(4) Urinary tract infection (by urine analysis).
(5) Patients with known SLE.
(6) Familial Mediterranean fever (FMF) (either known cases or history of recurrent abdominal pain, chest pain, and joint pain).
(7) Drugs causing proteinuria (e.g. analgesics).
(8) History of tumors, radiotherapy, and chemotherapy.
(9) Body temperature above 37.2°C by the thermometer.

Methods

This is a cross-sectional study that has been approved by the ethical committee of the Main University Hospitals, where all participants were recruited from Alexandria Main University Hospitals inpatient ward and outpatient clinics. After giving their signed informed consent, they were subjected to full detailed history taking, complete physical examination (vital signs, head and neck, chest, abdomen and pelvis, extremities and checking for lymph nodes) with the assessment of BMI, and were asked to refrain from strenuous exercise one day before sampling, and to fast for 12 h before sampling for the necessary laboratory investigations [complete blood count, erythrocyte sedimentation rate, glycosylated hemoglobin, total serum cholesterol, high-density lipoprotein, low density lipoprotein (LDL), serum triglycerides, blood urea, serum creatinine, alanine

aminotransferase, aspartate aminotransferase, complete urine analysis, UACR [11], and urinary PCX) [12] to assess the inclusion and exclusion criteria. A total of 169 individuals were recruited and thoroughly assessed for inclusion and exclusion criteria, and eventually only 80 patients met the inclusion criteria and were included in the study.

Urinary podocalyxin

It was measured using human PCX ELISA Kit (Glory Science Co. Ltd, Del Rio, Texas, USA, www.glorybioscience.com).

Principle

The kit is used for the quantitation of PCX in the sample. We adopted purified human PCX antibody to coat microtiter plate, to make solid-phase antibody, and then we added PCX to wells and combined PCX antibody with labeled HRP to form an antibody–antigen–enzyme–antibody complex. After washing completely, we added TMB substrate solution, and when it becomes blue color at HRP enzyme-catalyzed stage, the reaction is terminated by the addition of a stop solution, and the color change is measured at a wavelength of 450 nm. The concentration of PCX in the samples is then determined by comparing the optical density of the samples to the standard curve.

Detection range

Detection range of the kit is 0.26–1500 pg/ml.

It is to be noted that PCX and urinary albumin/creatinine ratio have been collected from the same sample of each patient.

Statistical analysis of the data [13]

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA) [14] Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, SD, and median. The significance of the obtained results was judged at the 5% level.

The used tests were Student *t* test, *F* test (analysis of variance), Pearson coefficient, Mann–Whitney test, Kruskal–Wallis test, Spearman coefficient, and receiver operating characteristic (ROC) curve, where it is generated by plotting sensitivity (TP) on Y axis versus 1-specificity (FP) on X axis at a different cutoff values. The area under the ROC curve (AUC) denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance, and area ~100% is the best performance for the test. The ROC curve allows also a comparison of performance between two tests. Moreover, we used sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and regression analysis.

Results

Table 1 shows the demographic and demonstrative data with the comparison between groups according to the different parameters, where comparison of all groups with UACR and PCX was statistically significant, with *P* values of less than 0.001 and 0.027, respectively.

Comparison of UACR levels between groups A and BI and between groups A and BII was statistically significant, with *P* value less than 0.001 for both, whereas between group BI and BII was not, with *P* value of 0.254. Comparison of PCX levels between group A and both groups BI and BII was not statistically significant, with *P* values of 0.539 and 0.109, respectively, whereas between group BI and BII was significant, with *P* value of 0.022, as shown in Fig. 1.

By using ROC analysis, urinary ACR showed that it can statistically differentiate between group A and either of group BI or BII at the cutoff point of 30, with 77% sensitivity and 100% specificity, with PPV 100% and NPV 81.6% [AUC 0.929, 95% confidence interval (CI) 0.008–0.133 and $P<0.001$], and it also can differentiate between groups BI and BII but at the cutoff point of 17, with 90% sensitivity and 82.5% specificity with PPV 83.7% and NPV 89.2% by (AUC 0.703, 95% CI 0.528–0.878 and $P<0.031$). However, PCX showed that it can statistically differentiate between groups BI and BII at the suggested cutoff point of 285 pg/ml with 52% sensitivity and 45% specificity with low PPV 48.8% and NPV 48.6% (AUC 0.749, 95% CI 0.590–0.907 and $P=0.008$), but it cannot statistically differentiate between group A and neither of group BI nor BII, as it was 100% positive in all patients of group A, as shown in Figs 2 and 3, with *P* value of 0.6.

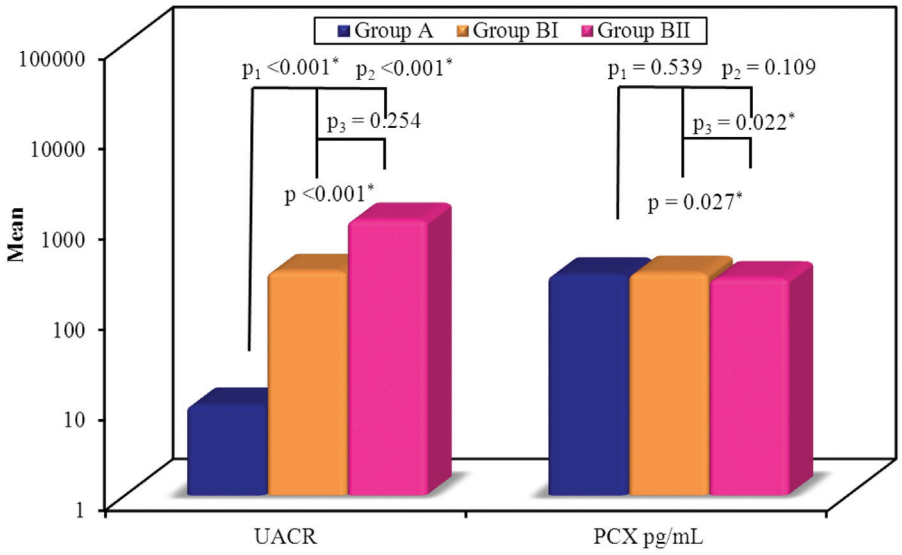
There are too many factors affecting UACR and PCX either positively or negatively but most of them are statistically insignificant. Further assessment by regression analysis using univariant and multivariate models showed that only creatinine and GFR were significantly correlated to UACR, whereas LDL and systolic blood pressure were significantly correlated to PCX as shown in Table 2.

Table 1 Demographic and descriptive data with comparison between the different groups according to different parameters

	Total (*N*=80)	Group A (*N*=40)	Group BI (*N*=24)	Group BII (*N*=16)	*P* value	Significance
Age (years)	51.79±7.14	50.40±7.54	52.42±6.94	54.31±5.84	0.158	NS
Sex [*n* (%)]						
Male	24 (30)	12 (30)	6 (25)	6 (37.5)	0.700	NS
Female	56 (70)	28 (70)	18 (75)	10 (62.5)		
BMI (kg/m^2)	33.68±5.72	32.65±4.23	33.46±6.89	36.57±6.39	0.064	NS
Overweight (25–<30)	28 (35)	15 (37.5)	10 (41.7)	3 (18.8)	0.076	NS
Obese (30–<35)	23 (28.8)	15 (37.5)	5 (20.8)	3 (18.8)		
Severely obese (35–<40)	18 (22.5)	8 (20.0)	6 (25.0)	4 (25.0)		
Morbidly obese (>40)	11 (13.8)	2 (5.0)	3 (12.5)	6 (37.5)		
Waist circumference (cm)	114.98±12.07	112±7.71	117±17.08	119.5±10.72	0.072	NS
Cholesterol (mg/dl)	190 (94–541)	186.5 (94–248)	201.5 (95–458)	183.5 (94–541)	0.279	NS
TG (mg/dl)	165.5 (63–1117)	155 (63–300)	176 (70–615)	181.5 (70–1117)	0.234	NS
LDL (mg/dl)	118.5 (38–368)	118.5 (38–178)	123.5 (43–368)	100 (48–297)	0.510	NS
HDL (mg/dl)	41 (20–388)	41.55 (25–62)	41.50 (27–388)	38.35 (20–64)	0.308	NS
HbA1c%	9.35±2.51	8.93±2.16	10.40±2.65	8.84±2.76	0.046*	S
FBS (mg/dl)	191 (65–450)	195.5 (65–450)	226 (99–436)	153.5 (66–317)	0.021*	S
ACEi/ARBS		0	16 (66.7)	3 (18.8)	<0.001*	S
Systolic blood pressure (mmHg)	147.19±21.97	157.1±22.78	140.4±17.06	132.5±13.42	<0.001*	S
Diastolic blood pressure (mmHg)	85.56±9.65	88.12±10.04	84.58±9.43	80.63±6.80	0.024*	S
Creatinine (mg/dl)	0.80 (0.50–3.80)	0.70 (0.50–1.17)	0.75 (0.50–1.17)	1.70 (1.30–3.80)	<0.001*	S
UACR	19.15 (1.10–5888)	7.90 (1.10–29)	50.45 (5.4–2761)	288.50 (1.7–5888)	<0.001*	S
GFR (4MDRD) (ml/min/1.73 m^2)	91.25 (12.9–414)	96.30 (56.60–236.5)	93.75 (50.50–414)	34.25 (12.90–55.10)	<0.001*	S
PCX (pg/ml)	272.10±44.58	274±43.05	286±41.91	248±44.97	0.027*	S

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GFR, glomerular filtration rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant; UACR, urinary albumin/creatinine ratio. Qualitative data were expressed using number and % and were compared using χ^2 test. Normally distributed quantitative data were expressed using mean±SD and were compared using *F* analysis of variance test, whereas abnormal data were expressed using median (minimum–maximum) and were compared using Kruskal–Wallis test. *Statistically significant at *P* value less than or equal to 0.05.

Figure 1

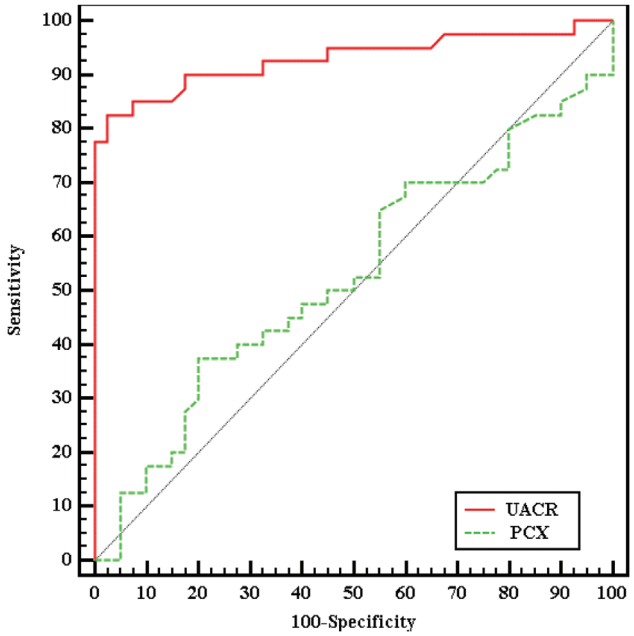


Comparison between the three studied groups according to different urinary biomarkers.

There was a negative correlation between UACR and PCX but statistically insignificant, with *P* value of 0.418.

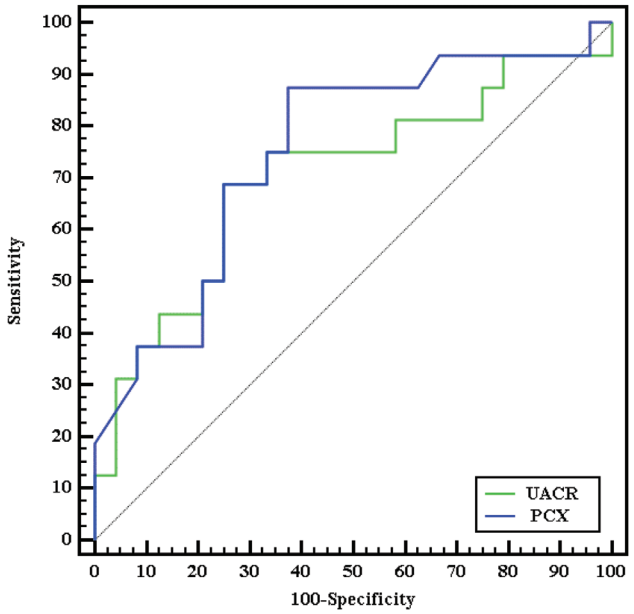
There is a nonsignificant correlation between BMI and the two biomarkers in all groups except UACR in group BII, which is significant, with *P* value of 0.017.

Figure 2



ROC curve for different parameters to diagnose group B from group A. ROC, receiver operating characteristic.

Figure 3



ROC curve for different parameters to diagnose group BII from group BI. ROC, receiver operating characteristic.

Table 2 Univariate and multivariate analyses for the parameters affecting urinary albumin/creatinine ratio and podocalyxin for total sample

		Univariate		Multivariate[a]	
		B (95% CI)	*P*	B (95% CI)	*P*
UACR	Cholesterol (mg/dl)	1.276 (−1.59 to 4.144)	0.379		
	ACEi/ARBs	54.35 (−426.9 to 535.66)	0.823		
	Systolic blood pressure (mmHg)	−5.35 (−14.65 to 3.95)	0.256		
	Cr (mg/dl)	731.5* (489.2–973.7)	< 0.001*	839.77* (545–1134.7)	<0.001*
	GFR (4MDRD) (ml/min/1.73 m^2)	−3.23* (−6.38 to −0.095)	0.044*	2.076 (−1.17 to 5.32)	0.207
PCX	Cholesterol (mg/dl)	0.136 (−0.002 to 0.273)	0.053		
	LDL (mg/dl)	0.222* (0.049–0.394)	0.013*	0.230* (0.066–0.394)	0.007*
	Systolic blood pressure (mmHg)	0.622* (0.187–1.058)	0.006*	0.642* (0.224–1.060)	0.003*

B: beta standardized coefficients. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, Confidence interval; GFR, glomerular filtration rate; LDL, low density lipoprotein; UACR, urinary albumin/creatinine ratio. [a]All variables with *P* value less than 0.05 was included in the multivariate. *Statistically significant at *P* value less than or equal to 0.05.

There is a significant relation between UACR and either ACEi or ARBs, with *P* value of 0.057, but no significant relation between them and PCX.

Discussion

Both obesity and metabolic syndrome can affect the kidneys, leading to an ESRD, by different pathophysiologic mechanisms that alter the nephron structure (glomeruli, tubules, interstitium, and vessels) and function leading to what is known as podocytopathy which is presented by proteinuria [7,8,10].

Podocytopathy is diagnosed either directly by biopsy and using light microscopy, electron microscopy, and immune staining or indirectly through serum and urinary biomarkers such as PCX, nephrin, podocin, CR1, CD80, synaptopodin, and many others biomarkers. So, we are searching if PCX can be used as an early biomarker for the diagnosis of podocytopathy.

The present study showed that UACR can statistically differentiate between group A and either of group BI or BII, with higher sensitivity and specificity than its differentiation between group BI and group BII. However, PCX showed the opposite of UACR, where it can statistically differentiate between groups BI and BII (with lesser sensitivity and specificity than UACR), but it cannot differentiate between group A

and neither of group BI nor BII, as it was 100% positive in all patients of group A.

The present study showed in Table 1 that *P* value for ACR was less than 0.001 and for PCX was less than 0.025, and UACR correlates better with estimated GFR. Moreover, it showed that as CKD progresses, level of UACR increases, whereas that of urinary PCX decreases, thus raising the possibility that UACR is better in following CKD progression than PCX which is explained by using ROC analysis where PCX can differentiate between CKD stages with higher *P* values than UACR but with lower sensitivity and specificity.

In other words, urinary PCX is found early in urine even before UACR could be detected, which means that it can be used as a sensitive biomarker for early diagnosis of podocytopathy even before the occurrence of albuminuria. On the contrary, its value in follow-up of patients with CKD is lesser than UACR.

In correlation with UACR in the present study, Krolewski [15] and also many other studies [16–18] showed that the change in albuminuria levels may not occur while CKD is progressing to ESRD, making the role of UACR in follow-up of patients with CKD controversial, which coincides with sensitivity and specificity shown by the ROC analysis to differentiate between BI and BII. This is explained by the two well-recognized pathways for DKD for both types 1 and 2 of diabetes mellitus: the albuminuric (the classical) and nonalbuminuric pathways. Both of them can progress to ESRD but the albuminuric patients progress faster than non-albuminuric patients. Moreover, those with albuminuric pathway can progress without a change in the albuminuria level. Currently, multiple researchers are studying the correlation between multiple factors and nonalbuminuric DKD progression especially dyslipidemia and BMI [18–22].

In addition, MacIsaac and Ekinci [22] and Radcliffe *et al.* [23] published the same pathways and recommended other factors (most of them are the consequences of DKD) for following up patients with DKD instead of UACR such as dyslipidemia, increased BMI, smoking, hypovitaminosis D, hemoglobin, glycosylated hemoglobin, blood pressure, and uric acid levels.

Keeping this in mind, the well-known role of UACR in early diagnosis and following up of DKD progression that was established for a long time and as shown in Persson and Rossing [24] should be questioned especially for those with nonalbuminuric pathway, and new surrogates are mandatory for early diagnosis and better follow-up, as UACR is no longer sufficient.

Podocytes are a critical component of GBM; their damage by repeated injuries leads to permanent loss (podocytopenia), as they are almost nonrenewable. Urinary PCX is an apical surface protein that is always present in the urine of almost all glomerular diseases as shown by Hara *et al.* [25] including minimal change nephrotic syndrome, hemolytic uremic syndrome, membranous nephropathy, membranoproliferative glomerulonephritis, Alport syndrome, lupus nephritis, poststreptococcal acute glomerulonephritis, Henoch-Schoenlein purpura nephritis, and IgA nephropathy but in early stages. However, the present study suggests that in advanced stages of CKD with reduction of GFR its level declines with the occurrence of more podocytopenia owing to the reduction of its expression by the shedded podocytes. This suggestion coincides with many other studies as of Lu *et al.* [26] where levels of urinary PCX are lower in advanced CKD or advanced glomerulosclerosis than those with normal GFR or less advanced or no glomerulosclerosis.

Lu *et al.* [26] and Hara *et al.* [27] concluded the same explanation as that of the present study, to the correlation between urinary PCX and renal functions which is the reduction of expression owing to shedded podocytes (functioning nephrons) with CKD progression, yet it is not significant in the present study, mostly owing to the small size.

LDL is secreted by macrophages and exacerbates atherogenesis-stimulated VSMC proliferation and plaque neovascularization [28]. PCX being expressed by vascular endothelium and being a pro-adhesive molecule that is associated with cell adhesion and migration [29,30], it is highly suggested that its level can be influenced by a vascular injury caused by atherosclerosis or hypertension.

In correlation with the present study, Shoji *et al.* [31] suggested that PCX can be used as a marker for atherosclerosis, where the idea of their research was based on the previously mentioned fact and hypothesis. Li *et al.* [32] observed a significant upregulation of PCX expression in the cells treated with oxidized-LDL which coincides with the strong positive correlation between PCX and LDL shown by the present study.

The present study showed that there is a nonsignificant correlation between BMI and the two biomarkers in all

groups, except urinary ACR in group BII, which is significant. In correlation with the present study, the DEMAND study by Rossi *et al.* [33] showed a tight correlation between changes in UACR level and with BMI and waist circumference changes which are independent of other metabolic risk factors over 1-year follow-up, which coincides with the finding of this study that UACR correlates only with BMI in group BII which has the highest BMI of all groups. Patients with overt diabetic nephropathy were excluded from the DEMAND study.

The present study could not find a significant relation between PCX and ACEi or ARBs, and also a barely significant relation between UACR and either ACEi or ARBs, with *P* value of 0.057.

Regarding UACR and in correlation with the present study, MICRO-HOPE [34], Brenner and Zagrobelny [35] and many other studies and meta-analysis since the 90s showed the tight correlation between UACR and ACEi and/or ARBs and their renoprotective mechanisms that are independent of blood pressure control but neither of them showed that they prevented the progression to ESRD [36–39]. Other studies showed a reduction of progression from 10 to 40% but there is no absolute prevention [40,41].

Albuminuria is an early marker of DN which is due to damage of the glomerular filtration barrier, which consists of endothelial cells, GBM, and podocytes. So, microalbuminuria means the involvement all of the three components in the injury. Podocyte injury or podocytopathy means cellular hypertrophy, effacement of the foot-process, detachment, and apoptosis. Added to these changes, the appearance of microvillous transformation on the apical cell surface of the podocyte leading to tip vesiculations that are shedded into the urine. The detection of PCX, being an apical surface protein, in urine makes it a marker of podocytopathy and a marker of the phenomenon of vesicular shedding that occurs in response to podocyte injury separable from the other two constituents of glomerular filtration barriers [42].

Hara *et al.* [27] provided evidence for this phenomenon in diabetic patients by demonstrating the presence of vesicles in urine by using anti-PCX antibodies. This phenomenon is not antagonized by the mechanism of action of ACEi and ARBs which explains why UACR is correlated to them, whereas PCX is not, and also explains why the presence of PCX is earlier than microalbuminuria.

Regarding PCX, Nakamura *et al.* [43] showed that urinary levels of PCX are greatly regressed by ACEi and ARBs equally after 3 months of follow-up compared with placebo and calcium channel blockers, but he did not include patients with CKD, as the present study, and his results may be obtained from the positive desirable effects of blood pressure control on diabetic nephropathy pathophysiology, by being a cohort study, not by the direct effect of RAAS blockers.

Fukuda *et al.* [44] showed that there are two sets of biomarkers assessing renal injury which are either cumulative biomarkers (that assess the cumulative effect of the events over time as 24-h urine volume, GFR, proteinuria, and blood pressure) or dynamic biomarkers (that assess the effect of the recent events as urinary proteins and podocytopathy markers). Both of them are complementary to each other and important for adequate assessment of the kidneys.

The present study concluded that PCX is a more sensitive dynamic biomarker than UACR but a less sensitive cumulative biomarker than UACR.

Conclusion

Although urinary PCX is a good sensitive biomarker for early diagnosis of podocytopathy even before the occurrence of albuminuria and is able to follow-up diabetic nephropathy patients with lesser sensitivity and specificity than UACR, UACR is still the gold standard for the diagnosis and follow-up of patients with diabetic nephropathy.

There are two limitations of this study: first, it was a cross-sectional study and there were time and follow-up limitations, where the markers were tested for their diagnostic adequacy and their relation with the medical condition of the patients at the time of sampling, so a longitudinal design is needed for better assessment of markers' adequacy, especially in disease progression follow-up, and the second, the small sample size may result in insignificance to some of the comparisons and correlations, which may be significant in larger sample size, and also may affect the sensitivity and specificity values shown by the ROC curves.

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Conflicts of interest
There are no conflicts of interest.

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