

Urinary cyclophilin A in Egyptian patients with type 2 diabetes and diabetic nephropathy: correlation with urine albumin/creatinine ratio

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Introduction

Albuminuria is commonly used to predict the onset and to follow the progression of diabetic nephropathy (DN), but it lacks both sensitivity and specificity in early stages.

Aim

We assessed urinary cyclophilin A (CypA) as a biomarker for the diagnosis of DN in Egyptian patients with type 2 diabetes.

Patients and methods

The study was conducted on 150 Egyptian participants aged 30–65 years; 125 (58 male individuals and 67 female individuals) patients with type 2 diabetes mellitus (diabetes duration >5 years) in different stages of DN and 25 age-matched and sex-matched healthy control participants comprised the study cohort. Estimated glomerular filtration rate and urine albumin/creatinine ratio (ACR) were assessed in all participants. Urinary CypA was measured in the morning specimen.

Results

Urinary CypA was significantly higher in patients with stage 2 DN, as compared with stage 1 patients ($P=0.02$) and the control group ($P=0.017$), with no significant change in urine ACR between stages 1 and 2 ($P=0.809$). Urinary CypA also showed a steady rise in DN stages 3, 4 and 5 ($P<0.001$). Urinary CypA had strong positive correlations with creatinine and urine ACR and a strong negative correlation with estimated glomerular filtration rate in patients with DN ($P<0.001$ for all).

Conclusion

We suggest that urinary CypA is a good biomarker for early detection of DN in patients with type 2 diabetes. It starts to rise before urine ACR. It also correlates well with the progression of DN. A larger study is needed to confirm its superiority over urine ACR in the early stages of DN.

Keywords:

albumin/creatinine ratio, diabetic nephropathy, type 2 diabetes, urinary cyclophilin A

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Introduction

The prevalence of microvascular and macrovascular complications associated with diabetes mellitus (DM) is dramatically increasing. Kidney disease is far more common in people with DM than in those without DM [1]. Microvascular changes within the kidney often lead to chronic kidney disease, an entity referred to as diabetic kidney disease or diabetic nephropathy (DN) [2]. This disease is characterized by a distinct histopathological pattern of glomerular basement membrane thickening, mesangial matrix expansion, and nodular glomerulosclerosis in addition to arteriolar hyalinosis [3]. The earliest detectable clinical manifestation of diabetic kidney disease is microalbuminuria [urine albumin/creatinine ratio (ACR) 30–300 mg/g], and, in the absence of early intervention, ~50% of patients with established microalbuminuria will progress to macroalbuminuria (urine ACR >300 mg/g), which is associated with a tenfold higher risk of progression to

end-stage renal disease (ESRD) than that of patients with normal urine albumin excretion (urine ACR <30 mg/g) [4]. The incidence of microalbuminuria in patients with type 1 DM is around 30% and largely depends on medication compliance and blood glucose control [5]. The incidence of microalbuminuria in patients with type 2 DM is also around 30% but is usually associated with hypertension [6].

Currently, the severity of DN is determined according to the level of albuminuria, which lacks both sensitivity and specificity to detect the early stages of the disease. In addition, inflammation and tissue damage have

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already occurred by the time microalbuminuria is detectable. Furthermore, some DN patients with ESRD do not present with significant albuminuria [7]. Therefore, there is a motivation to develop other diagnostic and prognostic tests for DN based on new biomarkers that can provide earlier warning and more specific information on disease progression and its response to treatment.

Cyclophilin A (CypA) represents a promising diagnostic marker for DN [8]. CypA is an 18-kDa protein distributed in the cytoplasm and facilitates protein folding and trafficking in addition to T-cell activation. It also catalyzes the isomerization of peptide bonds at proline residues, which allows it to regulate many biological processes, such as intracellular signaling, transcription, inflammation, and apoptosis [9].

The concentration of CypA is relatively high in the kidney. Proximal tubular epithelial cells are reported to contain much more of CypA than any other kidney tissue. CypA is directly produced by normal kidney, and its level increases in urine with kidney damage. It is also secreted by monocytes in response to hyperglycemia. Therefore, urinary CypA level could be a suitable indicator for early diagnosis of DN [10]. The aim of this study was to assess the value of urinary CypA as a biomarker for early diagnosis of DN in patients with type 2 diabetes.

Patients and methods

Our study included 150 Egyptian participants aged 30–65 years, recruited from the diabetes and endocrinology clinic. One hundred and twenty-five (58 male individuals and 67 female individuals) patients had type 2 DM for more than 5 years and were divided into five groups according to stages of DN (each included 25 patients):

Stage 1: urine ACR less than 30 mg/g and glomerular filtration rate (GFR) more than 120 ml/min/1.73 m². Stage 2: urine ACR less than 30 mg/g and GFR 90–120 ml/min/1.73 m². Stage 3: urine ACR 30–300 mg/g and GFR 60–120 ml/min/1.73 m². Stage 4: urine ACR more than 300 mg/g and/or GFR 15–60 ml/min/1.73 m². Stage 5: GFR less than 15 ml/min/1.73 m² not on renal replacement therapy.

Twenty-five age-matched and sex-matched healthy participants were also included as a control group. Patients with infectious diseases, chronic inflammatory diseases, liver diseases, malignant diseases and those on immunosuppressive or renal replacement therapy were excluded from the study.

All study participants were subjected to full medical history and thorough clinical examination. Fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), serum creatinine and lipid profile were all assessed. Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease equation. Urine ACR was calculated, and urinary CypA was measured, using an ELISA kit, in a spot urine sample. The study was approved by Cairo University Ethical Committee and Review Board. All the patients and control participants who participated in the study provided written informed consents.

Statistical methods

Data were coded and entered using the SPSS version 25. Data were summarized using mean, SD, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were carried out using the nonparametric Mann–Whitney test. For comparing categorical data, χ^2 -test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were carried out using Spearman correlation coefficient. *P* values less than 0.05 were considered statistically significant.

Results

Urinary CypA was significantly higher in the patients' group, as compared with the control participants ($P<0.001$). It was also significantly higher in patients with DN stage 2, as compared with stage 1 patients ($P=0.02$) and the control group ($P=0.017$). Urinary CypA also showed a steady rise in DN stages 3, 4 and 5 ($P<0.001$). Urine ACR did not show any significant difference between patients with DN stage 1 and stage 2 ($P=0.809$), but it started to show a significant progressive increase in stages 3, 4 and 5. Moreover, no significant difference was found between either stage 1 or stage 2 and the control group with regard to urine ACR ($P=0.145$ and 0.078 , respectively). Univariate analysis showed that urinary CypA had significant correlations with age, BMI and HbA1c ($P=0.018$, 0.007 and 0.005 , respectively) in the patients' group. Urinary CypA had strong positive correlations with creatinine and urine ACR and a strong negative correlation with eGFR in patients with DN ($P<0.001$ for all). Clinical and laboratory data of the patients and control participants are summarized in Tables 1–3.

Table 1 Clinical and laboratory data of the patients and control participants

Variables	Control (n=25) (mean±SD)	Stage 1 DN (n=25) (mean ±SD)	Stage 2 DN (n=25) (mean ±SD)	Stage 3 DN (n=25) (mean ±SD)	Stage 4 DN (n=25) (mean ±SD)	Stage 5 DN (n=25) (mean ±SD)	P value
Age (years)	54.4±9.19	48.07±6.25	52.67±9.36	54.4±7.95	55.8±7.76	56.73±6.62	0.46
BMI (kg/m ²)	28.9±6.51	35.74±9.21	31.79±6.97	31.79±7.74	33.54±8.41	29.1±4	0.176
DM duration (years)	NA	10.4±4.4	8.33±3.87	10.87±4.07	11.8±5.8	11.87±5.72	0.285
FPG (mg/dl)	91.2±11.77	145.1±49.6	154.4±62.9	185.6±56.5	197.1±105.1	159.5±70.4	<0.001
HbA1c (%)	5.9±0.3	6.7±1.1	6.6±1.1	7.5±1.4	7.2±1.3	7.3±0.9	<0.001
Creatinine (mg/dl)	0.7±0.1	0.6±0.1	0.7±0.1	0.8±0.1	1.3±0.6	4.9±1.4	<0.001
eGFR (ml/ min/1.73 m ²)	120.8±9.4	127.3±9.1	96.4±8.6	87.8±8.1	53.9±13.8	10.9±2.2	<0.001
Cholesterol (mg/dl)	161.6±32.8	216.1±64.4	222.7±74.2	216.8±62.2	220.8±118.6	175.6±28.1	0.42
TG (mg/dl)	138.1±32.9	217.8±86.7	190.1±83.5	149.4±60.3	235.7±182.3	159.9±47.4	0.088
LDL (mg/dl)	85.6±21.5	119.8±51.6	139.4±61.4	126.9±40.8	115.4±54.4	107.3±26.5	0.48
U. ACR (mg/g)	11.1±3.2	18.4±3.7	18.1±5.7	106.7±40.4	1515.1±689.2	4677.3±3913.8	<0.001
U. CypA (ng/ml)	0.61±0.25	0.63±0.28	2.85±0.82	6.59±1.84	6.89±2.55	9.43±2.11	<0.001

DM, diabetes mellitus; DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; NA, not applicable; TG, triglycerides; U. ACR, urine albumin/creatinine ratio; U. CypA, urinary cyclophilin A.

Table 2 Comparison between laboratory data in patients with stages 1 and 2 diabetic nephropathy

Variables	Stage 1 DN (n=25) (mean ±SD)	Stage 2 DN (n=25) (mean ±SD)	P value
Creatinine (mg/ dl)	0.61±0.14	0.7±0.08	0.531
eGFR (ml/min/ 1.73 m ²)	127.33±9.15	96.4±8.63	<0.001
U. ACR (mg/g)	18.42±3.73	18.09±5.74	0.809
U. CypA (ng/ ml)	0.63±0.28	2.85±0.82	0.020

DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; U. ACR, urine albumin/creatinine ratio; U. CypA, urinary cyclophilin A.

Discussion

DN is one of the most common microvascular complications of diabetes, and it is considered a leading cause of ESRD. Early diagnosis and intervention may give an opportunity to halt the permanent renal damage caused by DN [11]. Although albuminuria is the most commonly used marker for the diagnosis and follow-up of DN, it is far from being ideal. Microalbuminuria is a relatively late manifestation of early-stage DN, and some patients may have renal pathological changes without microalbuminuria [12]. Albuminuria is also not specific for DN and can be detected in other non-DM-related nephropathies; as in congestive heart failure [13]. There is a real need to have a new diagnostic marker for DN that can provide early warning and specific information on disease progression.

Table 3 Correlation between urinary cyclophilin A and other clinical and laboratory parameters of the patients (univariate analysis)

Variables	P value
Age	0.018
BMI	0.007
DM duration	0.186
FPG	0.681
HbA1c	0.005
Creatinine	<0.001
eGFR	<0.001
Cholesterol	0.156
TG	0.424
LDL	0.141
U. ACR	<0.001

DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; TG, triglycerides; U. ACR, urine albumin/creatinine ratio.

This study showed that Egyptian patients with type 2 DM and DN had significantly higher urinary CypA levels, as compared with the control group. All patients had type 2 DM for more than 5 years and were divided according to stages of DN. In stage 1, kidneys become dilated with increased glomerular capillary hydrostatic pressure, but without any ultrastructure abnormality [14]. Stage 2 DN is a silent stage with no established useful marker for detection. However, hyperglycemic injury is initiated at this stage. The glomerular basement membrane becomes thicker, followed by an increase in mesangial volume, and interstitial expansion [15]. These structural changes do not

become significant until stage 3. If stage 2 DN could be detected early, it might be possible to intervene early and slow the disease progress.

Our study revealed that urinary CypA was significantly higher in patients with stage 2 DN, as compared with stage 1 patients and the control group. However, no significant difference in urinary CypA was noticed between stage 1 DN and the control group. Urinary CypA also correlated well with the progression of DN. These results agree with those of Tsai *et al.* [10] who reported that, in Chinese patients with DM, a significant difference in urinary CypA levels was found between all stages of DN, except stage 1, and the control group. They also reported that urinary CypA increased gradually with the progression of DN.

In this study, urine ACR showed no significant difference between stages 1 and 2 DN. Moreover, no significant difference was found between either stage 1 or stage 2 and the control group with regard to urine ACR. Hence, in our Egyptian patients with type 2 diabetes and DN, urinary CypA was a more sensitive marker that increased significantly earlier before microalbuminuria and correlated well with the progression of nephropathy. Our results also agree with those of Amer *et al.* [16] who reported significantly high levels of urinary CypA in diabetic patients with any degree of renal affection, as compared with healthy participants, even in the absence of albuminuria.

This study demonstrated that urinary CypA had a significant positive correlation with serum creatinine and a significant negative correlation with eGFR in the study group. Tsai *et al.* [10] found a significant negative association between urinary CypA and eGFR in Chinese patients with DN. They also reported that the concentration of urinary CypA increased by 0.030 ng/ml with each 1 ml/min/1.73 m² decrease in eGFR.

This study also showed that urinary CypA had significant correlations with age, BMI and HbA1c, but not with FPG. Tsai *et al.* [10] found no significant correlation between urinary CypA and both FPG and HbA1c. Ohtsuki *et al.* [17] also reported no significant correlation between plasma CypA and HbA1c in Japanese patients with coronary artery disease. However, Ramachandran *et al.* [18] reported a significant positive correlation between plasma CypA and both FPG and HbA1c in Indian patients with type 2 DM. They also found that plasma CypA levels were increased in patients with diabetes and coronary artery

disease and suggested that CypA has a role in accelerating vascular disease in type 2 DM.

The small number of patients is a major limitation of our study. A large randomized controlled trial is needed to confirm the superiority of urinary CypA over urine ACR as a biomarker for early diagnosis of DN. Studying the effect of medications such as angiotensin-converting enzyme inhibitors or angiotensin receptor blockers on the levels of urinary CypA in patients with DN, especially in the early stages, would also help to evaluate the value of such a new marker in the follow-up of those patients.

Conclusion

This study showed that urinary CypA levels increase early in the silent stage of DN before microalbuminuria. It also correlates well with the progression of DN in patients with type 2 DM. We suggest that urinary CypA is a good biomarker for early detection of DN in Egyptian patients with type 2 DM.

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Conflicts of interest

There are no conflicts of interest.

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