



Plasma Cyclophilin A as a Novel Biomarker in Chronic Nephropathy

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TBE and HMAEM managed the analysis of the study. Author HMAEM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Type 2 diabetes mellitus (DM) is the most common cause of end-stage renal disease. Albuminuria is the foremost commonly utilized marker to anticipate onset of diabetic nephropathy (DN) without sufficient affectability and specificity to identify early DN.

Aim: This study aimed to evaluate Plasma cyclophilin A (CypA) as a new biomarker for early DN.

Methods: This cross sectional study included 125 Egyptian subjects attending the out Patients Clinic of the Department of Internal Medicine, 10th of Ramadan city Health Insurance Hospital and divided into:-control group, patient with diabetic mellitus, patients with Diabetic nephropathy and patient with diabetic nephropathy and other complications. Patients were subjected to measurement of plasma cyclophilin A, FBS, HbA1C, serum creatinine, serum urea, serum uric acid, k, Na, serum phosphorus, Albumin:Creatinine Ratio, GFR, Chol, TG, LDL HDL, AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.

Results: Results showed that Cyclophilin A was significantly correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, D.BIL. Meanwhile, Cyclophilin A was negatively correlated with HA1C, K, GFR, HDL, ALB, TP, GLB and A/G ratio. At

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cut-off level ≥ 84.14 , cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy.

Conclusion: CypA can be used as an early marker for DN as we found early significant high levels of urinary CypA in diabetic patients with stage 2 DN even before the appearance of albuminuria.

Keywords: Plasma cyclophilin A; DN.

1. INTRODUCTION

Type 2 diabetes mellitus (DM) is the most common single cause of end-stage renal disease (ESRD) [1].

ESRD in nearly half of patients is due to diabetic nephropathy (DN), and these cases have the most exceedingly bad result compared to patients with other causes of ESRD. In spite of the fact that there are numerous novel drugs for DM, there are no particular healing medicines however for DN. Reasons for destitute result incorporate insufficient markers and the complicated components of DN [2].

Now, severity of this disease is decided agreeing to the levels of albuminuria. Albuminuria is the foremost commonly utilized marker to foresee onset and movement of DN clinically. In any case, this conventional marker for DN needs both affectability and specificity to identify early organizes of DN [3]. However, some DN patients with ESRD do not present with significant albuminuria [4-6].

There is lack of association between glomerular filtration rate (GFR) and albuminuria suggests that an alternative to this albuminuria-based staging system is needed. Some studies have noted the existence of pathological change before microalbuminuria [4].

Therefore, even if micro albuminuria can be regarded as the earliest manifestation of DN, it is possible that a new biomarker for DN exists. Recently, different markers of DN were reviewed [7,8] including fibroblast growth factor 23 [9], tubular markers [10] (kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, and liver-type fatty acid-binding protein [L-FABP]) [11], inflammatory markers (interleukin 6 [IL-6], IL-8, monocyte chemo attractant protein 1, and interferon-inducible protein) [12], urinary 8-hydroxy-2'-deoxyguanosine [13], serum cystatin C [14] and so on. Among these, genetic susceptibility almost always leads to irreversible DN, and detection of the clinical markers mostly occurs too late to diagnose and monitor the progression of DN. As such, it is crucial to find

an earlier and reliable marker for DN. Earlier diagnosis and intervention may provide an opportunity to stop the permanent damage caused by DN.

Cyclophilin A (CypA) is an 18-kDa protein with ubiquitous characteristics. It is mostly distributed in the cytoplasm and facilitates protein folding and protein trafficking. It also acts as a cellular receptor for cyclosporine A (CsA). The expression of CypA is relatively high in the kidney, where proximal tubular epithelial cells (PTECs) are reported to contain considerably more CypA than other kidney tissues [14]. With respect to kidney diseases, the majority of research has been on the cellular relationship between CypA and CsA, which is used as an immunosuppressant, and leaves behind its secreted form. This secreted CypA (sCypA) was reported to be correlated with cardiovascular disease (CVD), asthma, rheumatoid arthritis (RA), and lung and liver injury. sCypA has been suggested to be a potential biomarker and mediator in CVD [15].

In addition, sCypA is associated with inflammatory or infectious diseases such as RA, asthma, and periodontitis. Interestingly, sCypA was also detected in diabetic patients' plasma and was shown to be secreted by monocytes in response to hyperglycemia, indicating that sCypA could be a potential secretory marker in type 2 DM [16].

Furthermore, a relatively high expression level of CypA in normal kidneys [17] has led to speculation that sCypA may be associated with solid organ damage. As a product directly produced by kidney, urine could be best measure for renal injury detection.

So this study aimed to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

1.1 Aim of the Work

The study aimed to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

2. PATIENTS AND METHODS

2.1 Study Design

Cross sectional study, aiming to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

2.2 Study Setting

The study was carried out at Clinic of the Department of Internal Medicine, 10Th of Ramadan city Health Insurance Hospital.

All of the above laboratory investigation except serum cyclophilin A level were done by using fully-automated auto-analyzer Cobas c 501 (Roche Diagnostics, Mannheim, Germany). Serum cyclophilin A concentrations were measured by using an Enzyme-Linked Immunosorbent Assay (ELISA) kit provided by (Biotech Co., LTD).

2.3 Target Population

Diabetic patients attending the Out Patients Clinic of the Department of Internal Medicine, 10Th of ramadan city Health Insurance Hospital.

This study included 125 Participants who were divided into:-

- Group A: (control group) 20 healthy subjects whose age ranged between 30-50 years old were taken as control group.
- Group B: 20 patients with diabetic mellitus whose age ranged between 30-50 years old.
- Group C: 65 patients' Diabetic nephropathy whose age ranged between 30-50 years old.
- Group D: Diabetic nephropathy and other complications whose age ranged between 30-50 years old.

2.4 Inclusion Criteria

1. Patients were free from infectious disease,
2. Patients were free from inflammatory disease,
3. Patients were free from liver disease,
4. Patients were free from malignancy, and
5. All were nonsmokers.

2.5 Exclusion Criteria

1. Patients who took drugs for hypertension,
2. Patients who took drugs for DM,

3. Patients who took drugs for hyperlipidemia,
4. Patients who took drugs for hyperuricemia,
5. Patients who took drugs for CVD,
6. Patients who took drugs for hyperuricemia, and
7. Patients who took drugs for gout.

All patients were subjected to the following;

- a. Collection of demographic data as required in the attached sheet including age, occupation, anthropometric measurements of height, weight, waist circumference, and history of disease.
- b. Collection of morning urine samples in vacutainer cup and also collection of 10 venous blood samples from the overnight fasted 5 ml blood were collected on plain tubes and other 5 ml blood were collected on EDTA tubes by vacutainer system under complete aseptic conditions and HbA1c first done and the samples centrifuged for 10 min at 2,500 g within 30 min separated serum and plasma were stored at 20°C the measurement of plasma cyclophilin A concentration, serum fasting glucose, serum creatinine, serum urea N, serum uric acid, serum potassium K, serum sodium Na, serum phosphorus, Albumin:Creatinine Ratio, GFR concentration, serum cholesterol and serum triglyceride. AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.
- c. The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 23). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The following tests were used.

2.6 Descriptive Statistics

Mean, Standard deviation (\pm SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data.

Frequency and percentage of non-numerical data.

2.7 Analytical Statistics

ANOVA test of significance was used when comparing between means of more than two groups.

Post-hoc test after ANOVA for significance between each two groups.

Chi-Square test was used to examine the relationship between two qualitative variables.

Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.

Correlation analysis (using Pearson's method) to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.

- $r=0-0.19$ is regarded as very weak correlation
- $r=0.2-0.39$ as weak correlation
- $r=0.40-0.59$ as moderate correlation
- $r=0.6-0.79$ as strong correlation
- $r=0.8-1$ as very strong correlation

Regression model to predict an outcome from independent factors.

ROC curve for prediction of independent value effect on the outcome.

2.7.1 P- value: level of significance

- $P>0.05$: Non significant (NS).

- $P<0.05$: Significant (S).

3. RESULTS

Table 1 shows that A total of 125 subjects were enrolled in this study; their mean age was 55.86±10.4 years (range, 24–82 years), and there were 71 men and 54 women. Age, BMI, Duration of D.M, F.B.G, C.P.A, HBA1C, S. creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.BIL, and D.BIL were significantly higher in diabetic patients than non-diabetic control. Meanwhile, K, Ph, T.P, AG ratio and CPA were significantly lower in diabetic patients than in non-diabetic controls. Other parameters did not differ significantly between the diabetes group and non-diabetic controls.

Also shows that Age, BMI, Duration of D.M, F.B.G, C.P.A, HBA1C, S. creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides,

HDL, LDL, AST, ALT, ALB, T.BIL, D.BIL, K, Ph, T.P, AG ratio and CPA were significantly different between four groups.

Table 2 shows that Cyclophilin A was significantly correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, D.BIL. Meanwhile, Cyclophilin A was negatively correlated with HA1C, K, GFR, HDL, ALB, TP, GLB and A/G ratio. However, there were no significant correlations between Cyclophilin A and FBS, HA1C and A/G ratio.

Table 3 and Fig. 1 show that at cut- off level ≥ 84.14 , cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy.

4. DISCUSSION

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes and it is considered as a leading cause of end-stage renal disease since there are no specific treatments for it till now. Therefore earlier diagnosis and intervention may provide an opportunity to stop the permanent renal damage caused by DN [18].

In our study, we tried to find out the possibility of using the plasma cyclophilin A (CypA) as a new marker for diagnosis of diabetic nephropathy as early as possible.

Our study showed that there was a statistically significant difference in the level of plasma CypA between the three main groups ($P < 0.01$) being higher in G3 and G2 than the control (G1).

In agreement with Amer et al. study in which the CypA in GIII (diabetics with albuminuria DN) (6.01 ± 1.61 ng/ml) was statistically significant higher than in GII (diabetics without albuminuria) (1.69 ± 0.87 ng/ml, $t = 12.93$, $p < 0.001$) and in GI (control) (0.55 ± 0.14 ng/ml, $t = 18.55$, $p < 0.0001$). In GII the CypA was statistically significant higher than in GI ($t = 7.04$, $p < 0.01$) [19].

We also found that the level of plasma CypA was statistically significant higher in group D (105.5 ± 5.26 ng/ml) than in group C (84.14 ± 7 ng/ml, $p < 0.001$).

This is in agreement with Tsai et al. [20] study which was the first study to use CypA in early detection of DN. It was conducted on 120 subjects; 20 healthy control group and 20

diabetic patients in each stage of DN (5 stages). Samples were collected to determine the expression of CypA. They also treated mesangial (MES-13) and tubular (HK-2) cells with glucose or free radicals to observe the expression of secreted CypA in Western blot analysis. They found that the levels of CypA were higher in groups of DN than in normal one. There was a highly statistical significant difference ($p < 0.001$) in levels of CypA between all groups except between normal and stage 1 DN groups where there was no significant difference in level of CypA between them. The lowest levels of CypA was found in control and stage 1 DN, wherein the CypA increased gradually with progression of DN till it reached the highest levels in stage 5 DN (ESRD).

This is compatible with Tsai et al. [20] study which was conducted on 100 type 2 diabetic patients in the different five stages of DN and

clarified that by comparing with the control group, CypA indeed increased significantly in stage 2 DN and its increase persisted throughout the later stages. The increment was more significant with worsening DN stage. They confirmed that there was no significant difference in concentration of CypA between stage 1 DN and healthy control groups ($P = 0.117$).

However, there were statistically significant differences between stages 1 and 2 ($P = 0.012$), stages 2 and 3 ($p = 0.003$), stages 3 and 4 ($p < 0.001$), and stages 4 and 5 DN ($P = 0.005$).

In our study, cyclophilin A was significantly correlated with duration of diabetes mellitus ($r = 0.271$, $p = 0.003$). This emphasizes the role of CypA in producing micro and macrovascular complications induced by prolonged duration of hyperglycemia.

Table 1. Baseline characteristics among study groups

	Group A	Group B	Group C	Group D	p-value
Age(years)	36.2±4.5	41.7±5.2	47.5±2.3	47±2.8	<0.001* ¹
Sex					
Male	12(60%)	12(60%)	34(52.3%)	13(65%)	0.935 ²
Female	8(40%)	8(40%)	31(47.7%)	7(35%)	
BMI(Kg/m ²)	27.3±2.3	28.7±2.6	30.2±3.2	30±2.9	0.002* ¹
Duration of D.M	--	4.95±2.4	8.82±2.9	9.15±2.7	<0.001* ¹
F.B.G	89.05±8.6	151.67±17	167.55±31.8	178.45±29	<0.001* ¹
C.P.A	37.35±6	84.14±7	59.89±4.76	105.5±5.26	<0.001* ¹
HBA1C	4.95±0.29	9.06±0.96	9.64±2.03	9.16±0.69	<0.001* ¹
S. creatinine	0.82±0.13	1.01±0.15	4±2.45	6.22±1.95	<0.001* ¹
S. urea	34.75±3.77	40.3±4.76	122.46±39.9	157.6±28.3	<0.001* ¹
UR.A	4.34±0.63	5.9±0.95	7.3±0.75	8.1±0.58	<0.001* ¹
Na	4.36±0.39	4.2±0.38	5.3±0.58	5.7±0.49	<0.001* ¹
K	141.95±2.96	140.4±2.3	133±4.9	123.4±5.88	<0.001* ¹
Ph	3.7±0.48	3.69±0.48	3.77±0.37	5.58±0.37	<0.001* ¹
ACR	10.9±1.9	21.6±3.41	324.12±328.3	3071.9±3241.4	<0.001* ¹
GFR	128.97±27	110.5±8.8	20.88±10.3	10.63±4.5	<0.001* ¹
Cholesterol	169.15±13.4	170.9±12.1	181±20.5	238.7±27.2	<0.001* ¹
Triglycerides	127±8.9	156.2±15.9	146.5±16.1	191.2±38.6	<0.001* ¹
HDL	47.8±2.6	40.8±3.35	44.8±5.94	36.4±3.49	<0.001* ¹
LDL	96.9±13.1	99.2±11.78	107.6±19.75	170.3±22.45	<0.001* ¹
AST	26.85±3.37	37.15±7.7	37.17±6.8	64.7±13.15	<0.001* ¹
ALT	26.85±2.62	36.9±6.9	36.7±5.9	37.5±14.9	<0.001* ¹
ALB	4.05±0.2	4±0.20	3.8±0.23	3.5±0.19	<0.001* ¹
T.BIL	0.61±0.07	0.68±0.09	0.72±0.07	0.87±0.04	<0.001* ¹
D.BIL	0.16±0.19	0.18±0.02	0.18±0.02	0.22±0.02	<0.001* ¹
T.P	7.03±0.21	7.01±0.22	6.8±0.28	6.2±0.31	<0.001* ¹
GLB	3.02±0.17	3.02±0.17	2.95±0.16	3±0.23	<0.001* ¹
AG ratio	1.76±0.21	1.35±0.1	1.28±0.15	1.32±0.17	<0.001* ¹
C.P.A	37.35±6	59.89±4.76	84.14±7	105.5±5.26	<0.001* ¹

ANOVA test; 2. Chi-square test

*Statistical significant when p-value <0.05

Table 2. Correlations between CPA levels and other parameters in patients with diabetes

	C.P.A	
	r	P-value
Duration of DM	0.271	0.003*
F.B.S	0.137	0.136
HBA1C	-0.051	0.581
CR	0.596	<0.001*
Urea	0.681	<0.001*
UR.A	0.626	<0.001*
Na	0.611	<0.001*
K	-0.708	<0.001*
Ph	0.692	<0.001*
ACR	0.484	<0.001*
GFR	-0.782	<0.001*
Chol	0.595	<0.001*
TG	0.532	<0.001*
HDL	-0.231	0.018*
LDL	0.608	<0.001*
AST	0.589	<0.001*
ALT	0.604	<0.001*
ALB	-0.563	<0.001*
T.BIL	0.576	<0.001*
D.BIL	0.362	<0.001*
T.P	-0.604	<0.001*
GLB	-0.260	0.007*
A/G ratio	-0.089	0.364

Pearson correlation test

*Statistical significant when p-value <0.05

Table 3. Validity of C.P.A for diabetic nephropathy

	AUC	Sensitivity	Specificity	Cut-off value
CPA	0.79	91%	62%	84.14

This table showed that at cut-off level ≥84.14, cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy

In agreement with Amer et al. study which found a significant positive correlation between CypA levels and the duration of diabetes in the three diabetic groups. There was noticed that the correlation was stronger in GIIIb which had the longest duration of diabetes (16.40±4.56 yrs, r=+0.97, p <0.001) than in GIIIa (13.00±4.14 yrs, r=+0.90, p <0.001) which in turn had a stronger correlation than in GII (7.29±6.17 yrs, r=+0.67, p <0.01) [19].

This is in contrast with Tsai et al. study that showed no significant correlation (p=0.957) between the level of CypA and duration of diabetes [20]. This discrepancy may be due to that in our research by chance the longest durations of diabetes were presented in group D.

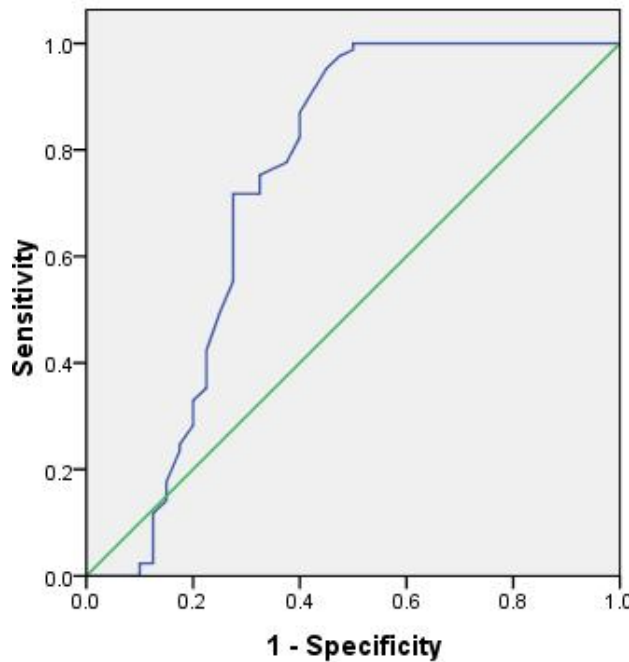
So the significant elevations in plasma CypA may be contributed by the severity of renal

damage which was more aggressive in group D and not by duration of diabetes itself.

In the present study, we found that there was no significant positive correlation between plasma CypA and both of FBS and HA1C in all studied groups. Although these correlations between urinary CypA levels and FPG and HbA1c are non-significant, they were much higher in group C and group D than group A and B. This illustrates that higher levels of hyperglycemia induced higher levels of plasma CypA which may allow us to use CypA level also as a marker for diagnosis of DM.

Similar to Amer et al. study, in which there was no significant positive correlation between plasma CypA and both of FPG and HbA1c in all studied groups [19].

ROC Curve



Diagonal segments are produced by ties.

Fig. 1. ROC curve of C.P.A for diabetic nephropathy

This is in consistency with Tsai et al. [20] study that showed no significant correlation between urinary CypA and each of FPG ($p= 0.898$), and HbA1c ($p= 0.686$) as well as Ramachandran et al. [21] study which exhibited no significant correlation between CypA and HbA1c($p= 0.232$).

In our study, correlations between plasma CypA and other renal parameters (sCr, ACR, eGFR) were done in attempt to explain the presence of higher levels of plasma CypA with the more severe stages of DN.

Similar to Amer et al. study who found that there was a significant positive correlation between CypA and sCr in GII ($r= +0.39$, $p< 0.05$), while there was a highly significant positive correlations between CypA and sCr in both GIIIa ($r= +0.89$, $p< 0.001$) and GIIIb ($r=+0.99$, $p<0.001$). While there was no significant positive correlation between urinary CypA and normal sCr in GI ($r=+0.07$, $p= 0.73$) [19].

This indicates that CypA levels increase proportionally with the elevation in sCr. As CypA

levels were low in GI who had normal sCr levels and it started to increase significantly in patients with DN. The increment of CypA became more significant in group 3 (sCr 4 ± 2.45 mg/dl) whilst the highest significant increase in CypA was in group 4 who had the highest levels of sCr (6.22 ± 1.95 mg/dl).

Our study is compatible with Tsai et al. who studied the urinary CypA as a new marker of DN. They clarified that there was a significant positive correlation ($p= 0.037$) between urinary CypA and sCr. In addition, it demonstrated that the concentration of urinary CypA increased by 0.395 ng/ml for each 1 mg/dl increase in sCr. It put a constant equation illustrating the relation between urinary CypA and sCr [20].

In our study, there was a highly significant negative correlation between CypA levels and eGFR in the diabetic groups (group B, C, and D).

Similarly, Amer et al. research established that there was a highly significant negative correlation between CypA levels and eGFR in the diabetic groups and this correlation became

more significantly higher while the decrease in eGFR became more advanced. ¹⁹It was noticed that this correlation was higher in GIIIb (eGFR 41.26±16.37 ml/min/1.73 m², r= -0.98, p< 0.001) than in GIIIa (eGFR 72.12±22.48 ml/min/1.73 m², r= -0.90, p< 0.001) which in turn higher than in GII (eGFR 96.59±21.90 ml/min/1.73 m², r= -0.76, p<0.01). Whereas there was no significant negative correlation between urinary CypA and normal eGFR in GI (e GFR 102.98±8.09 ml/min/1.73 m², r= -0.07, p= 0.71). So we concluded that the CypA only significantly increased with renal affection and decrease of eGFR.

This is in agreement with Tsai et al. study that proved the presence of significant negative association between urinary CypA and eGFR in DN patients (p= 0.013) [20].

Also, they found that the concentration of urinary CypA increased by 0.030 ng/ml with each 1 ml/min decrease in eGFR and they established an equation which illustrated the correlation between Urinary CypA and eGFR (CypA= 5.270+GFR*-0.030).

Besides, the study showed that there was a trend of higher urinary CypA in the group with GFR less than 60 ml/min/1.73 m² as compared to the group with GFR more than 60 ml/min/1.73 m² (p< 0.060).

Our results showed that there was a highly significant positive correlation between CypA and the severity of albuminuria (ACR) in diabetic groups.

In agreement with Amer et al. study which reported that there was a highly significant positive correlation between CypA and the severity of albuminuria (ACR) in GIII. These positive associations were more significant in GIIIb (ACR 1267.53±688.20 mg/g, r= +0.98, p< than in GIIIa (ACR 226.83±74.96 mg/g, r= +0.93, p<0.001). While there was no significant positive correlation between urinary CypA and normal ACR as in GI (ACR 9.29±1.19 mg/g, r= +0.24, p= 0.21) and in GII (ACR 20.96±4.25 mg/g, r= +0.25, p=0.18) [19].

Our results are fit with that of Tsai et al. study that illustrated that; there was a statistically significant difference (p= 0.007) in the levels of urinary CypA between both proteinuric and nonproteinuric patients where in non-proteinuric the concentration of urinary CypA decreased by 3.095 ng/ml. They also proved that when ACR

increased by 1 mg/g, the concentration of urinary CypA increased by 0.030 ng/mL and they established an equation to link between them (CypA=2.461+ACR*0.001) [20].

In our study, at cut-off level ≥84.14, cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy.

From the previous results we can conclude that CypA level is strongly correlated with the degree of renal affection and its level started to increase significantly in complications and continued to increase proportionally with the progression of DN.

This confirms the presence of strong alternative relation between CypA and DN. To conclude, although the albuminuria based system is the most common used marker for the diagnosis and follow up the progression of DN, it is far from ideal for a number of reasons. First, increased albuminuria is actually a relatively late manifestation of early- stage DN, so it is not sensitive enough to detect early stages of DN. Second, some patients have renal pathological changes without micro albuminuria. Finally, albuminuria is not specific enough for DN because it can be detected in other non-DM related nephropathy, such as retinopathy and congestive heart [22].

In addition, due to that either GFR-based or albuminuria-based classifications of DN correlated significantly with urinary CypA.

When comparing different stages of DN or Chronic kidney disease (CKD), there was only a trend of higher CypA in higher CKD stages, but truly statistically significant difference existed among the different DN stages. This finding supports the notion that CypA is better correlated using the albuminuria-based classification, which is the better and earlier detection method for monitoring DN in clinical practice.

This will enable us to detect stage 2 DN early, so intensive blood sugar monitoring, timely diet restriction and exercise education would be useful to avoid further silent deterioration of DN.

5. CONCLUSION

CypA was higher in diabetics with macroalbuminuric DN than those with microalbuminuric DN who in turn had higher levels of urinary CypA than diabetics with

normoalbuminuric DN. CypA had a positive correlation with serum creatinine, urinary albumin creatinine ratio and duration of diabetes, while it had a negative correlation with estimated glomerular filtration rate.

CypA can be used as an early marker for DN as we found early significant high levels of urinary CypA in diabetic patients with stage 2 DN even before the appearance of albuminuria.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Hostetter TH. Prevention of end-stage renal disease due to type 2 diabetes. *N Engl JMed*. 2001;345:910–912.
- Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: An update. *Vascul Pharmacol*. 2013;58:259–271.
- Halimi JM. The emerging concept of chronic kidney disease without clinical proteinuria in diabetic patients. *Diabetes Metab*. 2012;38:291–297.
- Tramonti G, Kanwar YS. Review and discussion of tubular biomarkers in the diagnosis and management of diabetic nephropathy. *Endocrine* 2013;43:494–503.
- Kramer HJ, Nguyen QD, Curhan G et al. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 2003;289:3273–3277.
- MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, et al. Nonalbuminuric renal insufficiency in type 2 diabetes. *Diabetes Care*. 2004;27:195–200.
- Kamijo-Ikemori A, Sugaya T, Kimura K. Novel urinary biomarkers in early diabetic kidney disease. *Curr Diabetes Rep*. 2014;14:513.
- MacIsaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis* 2014;63(2 Suppl 2):S39– S62.
- Titan SM, Zatz R, Gracioli FG, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin J Am Soc Nephrol*. 2011;6:241–247.
- Nielsen SE, Andersen S, Zdunek D et al. Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy. *KidneyInt*. 2011;79:1113–1118.
- Tramonti G, Kanwar YS. Tubular biomarkers to assess progression of diabetic nephropathy. *Kidney Int*. 2011;79:1042–1044.
- Wolkow PP, Niewczas MA, Perkins B et al. Association of urinary inflammatory markers and renal decline in micro albuminuric type 1 diabetics. *J Am Soc Nephrol*. 2008;19:789–797.
- Hinokio Y, Suzuki S, Hirai M et al. Urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy. *Diabetologia*. 2002;45:877–882.
- Krolewski AS, Warram JH, Forsblom C et al. Serum concentration of cystatin C and risk of end-stage renal disease in diabetes. *Diabetes Care*. 2012;35:2311–2316.
- Satoh K, Shimokawa H, Berk BC, Cyclophilin A. Promising new target in cardiovascular therapy. *Circ J*. 2010;74:2249–2256.
- Nigro P, Pompilio G, Capogrossi MC. Cyclophilin A: A key player for human disease. *Cell Death Dis* 2013;4:e888.
- Ramachandran S, Venugopal A, Kutty VR et al. Plasma level of cyclophilin A is increased in patients with type 2 diabetes mellitus and suggests presence of vascular disease. *Cardiovasc Diabetol*. 2014;13:38.
- Mora-Fernández C, Domínguez-Pimentel V, de Fuentes MM et al. Diabetic kidney disease: From physiology to therapeutics. *J. Physiol*. 2014;592:3997
- Amer Hanan, Mohamed Ali et al. The role of urinary cyclophilin A as a new marker for diabetic nephropathy. *The Egyptian Journal of Hospital Medicine*. 2018;70(9):1431- 1439.
- Tsai S, Su CW, Wu MJ et al. Urinary Cyclophilin A as a New Marker for Diabetic Nephropathy A Cross-Sectional Analysis of Diabetes Mellitus. *Medicine*. 2015;94(42):e1802.
- Ramachandran S, Venugopal A, Kutty VR et al. Plasma level of cyclophilin A is

increased in patients with type 2 diabetes mellitus and suggests presence of vascular disease. Cardiovascular Diabetology. 2014;13:38.

22. Zachwieja J, Soltysiak J, Fichna P et al. Normal-range albuminuria does not exclude nephropathy in diabetic children. *Pediatr Nephrol.* 2010;25:1445–1451.

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