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Association between Urinary Cyclophilin A and Diabetic Nephropathy in Children with Type 1 Diabetes Mellitus

Dina F. Khamis[1*], Amina M. Abdel Wahab[2], Hanan H. Omar[3], Zeinab A. Mohammed[4]

[1]*Neonatology Unit, MetGhamr Central Hospital, Egypt*
[2]*Department of Pediatrics, Faculty of Medicine, Suez Canal University, Egypt*
[3]*Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Egypt*

Abstract

Background: **Diabetes is a group of metabolic diseases marked by hyperglycemia caused by insulin secretion, action, or both. Diabetes chronic hyperglycemia is associated with long-term damage, dysfunction, & failure of numerous organs, most notably eyes, kidneys, nerves, heart, & blood vessels.** ***Aim and objectives:*** **This research aimed to evaluate the association between Urinary Cyclophilin A & diabetic nephropathy in children& adolescents with Type 1 Diabetes Mellitus (T1DM).** ***Subjects and Methods:*** **Sixty children& adolescents were recruited consecutively from those attending diabetes outstudied case clinic in Suez Canal University Children's Hospital. Thirty years old & gender-matched children & adolescents were recruited as the control group.** ***Results:*** **Concerning urinary CyPA, we found that those with microalbuminuria had the highest urinary CyPA levels when compared to others. This was statistically significant (p<0.001). On performing post hoc analysis, we found that variation could be explained by the difference among group B included normoalbuminuric diabetic children and adolescents and group C included healthy children and adolescents (p<0.001), among group A included microalbuminuric diabetic children and adolescents &group C (p<0.001).** ***Conclusion:*** **Urinary CyPA can be used as an early indicator for Diabetic nephropathy because important great levels of urinary CyPA were detected in T1DM studied cases with DN before the presence of albuminuria.**

Keywords: Diabetes, juvenile, Cyclophyllin A, relationship

Introduction

Diabetes mellitus is a collection of metabolic illnesses characterized by prolonged hyperglycemia; it arises as an outcome of either a lack of insulin secretion, a lack of insulin action, or both(1). Progressive proteinuria, hypoalbuminemia, edema, hypertension, and chronic renal failure are all symptoms of diabetic nephropathy (DN), a kidney condition brought on by long-term hyperglycemia. DN results in nonenzymatic glycation reactions of proteins and peptides & production of advanced glycation end-products, which are linked to inflammation and damage to the renal

*Corresponding Author: faroukdina718@gmail.com

glomeruli[2]. DN is the main side effect of diabetes and a significant contributor to end-stage renal failure (ESRD). In type 1 diabetes, microalbuminuria is a risk factor for cardiovascular events and nephropathy and is regarded to be an early indicator of DN in clinical practice[3]. However, there are several limits to albuminuria that need to be considered. First of all, not every person with proteinuria will eventually acquire progressive renal impairment[4]. Second, the start of microalbuminuria frequently occurs before advanced DN-related histological alterations. Normoalbuminuria is present in a sizable portion of diabetic patients with DN[5]. Third, the high degree of confounding factors that affect urine albumin excretion, such as stress, physical activity, infections, fever, menstrual bleeding, and severe hyperglycemia[6]. Inflammatory markers like interleukin 6, interleukin 8, monocyte chemoattractant protein 1, and interferon-gamma inducible protein, tubular markers like kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, & liver-type fatty acid-binding protein, urinary 8-hydroxy-20-deoxyguanosine, Cystatin C, & urinary cyclophilin A; and new biomarker are used for recognition of diabetic nephropathy[7]. It is widely known that CyPA, a growth factor released in response to oxidative stress, acts as a mediator of tissue damage brought on by inflammation and oxidative stress[8]. In comparison to other kidney tissues, proximal tubular epithelial cells in the kidney have a comparatively high concentration of CyPA. CyPA is directly produced by healthy kidneys, hence any kidney impairment will result in an increase in the level of CyPA in urine. Therefore, as monocytes secrete it in response to hyperglycemia, urine CyPA level would be the best marker for an early diagnosis of DN[9]. There is insufficient data on the relationship between urinary CyPA & DN in children with T1DM. As a result, the present research will be carried out to assess this relationship.

Subjects and Methods

Research Setting and Study Population

This research was an observational controlled study conducted at a Diabetic outstudied case clinic in Suez Canal University Children's Hospital, Ismailia, Egypt. The study was conducted from May 2021 to December 2021. The patients were randomly selected according to the ISPAD Guidelines 2014[10]; where screening for DN in children with T1DM starts at years old of ten, or at the onset of puberty if earlier, with diabetes time of two to five years. Our target population was children& adolescents with (type 1D who were separated into three groups: Group A (n=30) included microalbuminuric diabetic children and adolescents. Group B (n=30) included normoalbuminuric diabetic children and adolescents. Group C (n=30) included healthy children and adolescents.

Inclusion criteria

Type 1 diabetic children of both genders, 10yr of age or older (up to18) or at the onset of puberty if this is earlier, with two-five years of diabetes time. Diabetic children on intensive insulin therapy.

Exclusion criteria

Children with any of the following were excluded: congenital or acquired kidney disease, thyroid dysfunction, history of recent infection or diabetic ketoacidosis, or those who received nephrotoxic drugs or glucocorticoids.

Methods

A- Full history taking

Years old, gender, presence of parental consanguinity, age at onset of diabetes, family history, comorbidities, associated

complications, drug history, years old at diagnosis of diabetes and level of plasma glucose at the duration of diagnosis, duration of the disease and history of acute complications or vascular complications and Treatment of T1DM. Insulin dose (unit/kg/day).

B- Complete clinical examination

General examination. Vital data temperature, heart rate, respiratory rate & blood pressure were recorded**.** Standard physical tests, such as weight & height, were performed using standard methods. Body mass index was calculated as weight (kg)/height (m2), followed by Body mass index standard deviation score for years old & gender using Egyptian growth charts[11]**.** Tanner categories of breast development in women & genital advancement in men were used to decide pubertal development for all research participants [12]**.** Blood pressure was measured using a standard mercury manometer & outcomes were plotted on blood pressure curves based on years old, gender, & height centiles. Hypertension was defined as having average systolic blood pressure & diastolic blood pressure that is larger than the ninety-fifth percentile for years old, sex, & height for at least 3 measurements[13]**.**

C- Investigations

1- Blood sampling

Venous whole blood was withdrawn from everyone for routine investigations (i.e. complete blood count, random blood sugar, serum electrolytes, and lipid profile). HbA1c by affinity chromatographic methods, Serum creatinine by enzymatic creatinine assay. The estimated glomerular filtration rate (eGFR; mL/min/1.73 m^2) was calculated using the following formula: Serum creatinine-based eGFR (eGFR-Cr) was calculated using the updated Schwartz formula[14]: eGFR-Cr = 0.413×height (cm)÷Serum Cr (mg/dL)

2- Urine sampling:

A clean catch mid-stream urine specimen was collected for urine analysis. Urinary creatinine (mg/dL) by Jaffe method. The albumin creatinine ratio is measured by dividing the amount of urine albumin by the amount of urine creatinine. Urine was collected in the morning & stored at 20C° throughout the duration of the assay.

Urinary Cyclophilin A

Urinary Cyclophilin A (CyPA) level was determined by ELISA following the manufacturer's instructions (Abcam, UK). The technique used a double antibody sandwich enzyme-linked immunosorbent assay. We added CyPA to the monoclonal antibody enzyme well that had already been pre-coated with CyPA monoclonal antibody enzyme, incubated it, then added CyPA antibodies labeled with biotin & blended with streptavidin HPR to establish an immune complex, incubated it again, & washed to remove uncombined enzyme. When we add chromogen solutions A, and B, the color of the liquid changes to blue, & when we add acid, the color changes to yellow. color chroma & concentration of Human Substance CyPA in samples were found to be positively correlated.

Results

Study population characteristics

The research included 90 subjects and was separated into three groups; 30 subjects each. Group A: diabetic studied cases with microalbuminuria. Group B: diabetic studied cases with normoalbuminuria. Group C: healthy controls. All groups were years old & gender matched with no important variance among them (p=0.206, p=0.185). Concerning the onset age of

DM; patients with microalbuminuria had developed type 1 DM earlier than others with normoalbuminuria as shown in Table 1 (p=0.014).

Table 1: Comparing among three tested groups according to demographic data

	Group A (n =30)		Group B (n =30)		Group C (n =30)		Test of Sig.	P
	No.	%	**No.**	%	**No.**	%		
Gender								
Men	21	70.0	14	46.7	17	56.7	χ^2= 3.370	0.185
Women	9	30.0	16	53.3	13	43.3		
Years old								
Min. – Max.	11 – 16		10 – 16		10.0 – 17.0		F= 1.609	0.206
Mean ± SD.	13.37 ± 1.47		13.37 ± 1.96		14.17 ± 2.44			
Age at DM onset								
Min. – Max.	eight – thirteen		6 – 13		–		t= 2.534*	0.014*
Mean ± SD.	10.53 ± 1.46		9.37 ± 2.06		–			
Insulin TDD								
Min. – Max.	0.50 – 1.50		0.50 – 1.50		–		t= 0.231	0.818
Mean ± SD.	1.18 ± 0.28		1.20 ± 0.28		–			
BMI Z-score								
Min. – Max.	-1.23 – 1.34		-1.20 – 1.40		-0.84 – 0.39		H= 6.155*	0.046*
Median (IQR)	-0.14 (-0.65 – 0.80)		0.13 (-0.27 – 0.69)		-0.37 (-0.57 – 0.17)			
Sig. among groups	p_1=0.209, p_2=0.220, p_3=0.013*							
SBP (mmHg)								
Min. – Max.	100.0 – 115.0		100.0 – 115.0		100.0 – 115.0		F= 1.292	0.280
Mean ± SD.	108.0 ± 5.96		106.17 ± 4.86		108.17 ± 5.17			

*SD: Standard deviation; F: F for ANOVA examination; t: Student t-test; H: H for Kruskal Wallis test, pairwise comparing among every two groups was completed using Post Hoc Test; p: p-value for comparison among tested groups; p_1: p-value for comparison among Group A & Group B; p_2: p-value for comparison among Group A & Group C; p_3: p-value for comparison among Group B & Group C; *****: important at p ≤ 0.05. Group A: Microalbuminuric diabetic children; Group B: Normoalbuminuric diabetic children; Group C: Healthy Children*

Concerning BMI Z score, patients with the microalbuminuric state had a greater range for BMI Z score when compared to others as shown in Table 1 (p=0.046). Post hoc analysis presented important variation between healthy controls and normoalbuminuric diabetic patients (p=0.013). BMI z score was slightly higher among those with microalbuminuria when compared with those with normoalbuminuria, with a statistically insignificant difference (p=0.209) (table 1). Concerning SBP, we found that there was a slight difference between all groups, which was statistically insignificant (p=0.280). Similarly, for Insulin TDD, we showed that studied cases with normoalbuminuria had slightly greater levels when compared with others, this was also statistically insignificant (p=0.818) (table 1). Urinary CyPA expression was more significantly present among diabetic patients when compared with the control group. This was important (p<0.001) as shown in Table 3. Concerning lipid profile, we found that despite triglyceride serum levels were high among those with normoalbuminuric diabetic state, this was statistically insignificant (p=0.630). On the other hand, total cholesterol serum levels were high

among those with microalbuminuria compared to others as shown in Table 4 (p<0.001). Similarly, for LDL, mean serum levels were significantly high among those with microalbuminuria compared to others as shown in the table 4 (p<0.001). Concerning HbA1c serum levels, studied cases with microalbuminuria had greater levels of HbA1c when compared with others (p<0.001).

Table 2: Comparing the tested groups according to laboratory data

	Group A (n =30)	Group B (n =30)	Group C (n =30)	F	p
Total cholesterol (mg/dL)					
Min. – Max.	128.0 – 183	123 – 166.0	111 – 160.0	35.850*	<0.001*
Mean ± SD.	164.47 ± 14.19	141.87 ± 14.10	134.17 ± 14.92		
Sig. among groups	p_1<0.001*,p_2<0.001*,p_3=0.102				
Triglycerides (mg/dL)					
Min. – Max.	46.0 – 132.0	61.0 – 123.0	58.0 – 105.0	0.464	0.630
Mean ± SD.	87.33 ± 26.38	86.37 ± 19.06	82.40 ± 16.28		
HDL (mg/dL)					
Min. – Max.	37 – 57	40 – 64.0	39 – 67	4.274*	0.017*
Mean ± SD.	46.30 ± 5.98	51.27 ± 7.57	51.13 ± 8.69		
Sig. among groups	p_1=0.032*,p_2=0.038*,p_3=0.997				
LDL (mg/dL)					
Min. – Max.	65.0 – 110.0	50.0 – 98.0	42.0 – 90.0	18.864*	<0.001*
Mean ± SD.	90.0 ± 14.0	79.27 ± 14.95	66.30 ± 15.89		
Sig. among groups	p_1=0.018*,p_2<0.001*,p_3=0.003*s				
HbA1c (%)					
Min. – Max.	7.10 – 8.80	6.40 – 8.10	4.60 – 5.40	366.095*	<0.001*
Mean ± SD.	8.07 ± 0.51	7.28 ± 0.54	5.00 ± 0.25		
Sig. among groups	p_1<0.001*,p_2<0.001*,p_3<0.001*				
Urinary albumin to creatinine ratio					
Min. – Max.	49.80 – 267.80	12.60 – 24.40	10.40 – 23.50	142.252*	<0.001*
Mean ± SD.	156.01 ± 63.24	18.61 ± 3.36	17.02 ± 4.13		
Sig. among groups	p_1<0.001*,p_2<0.001*,p_3=0.985				
Serum creatinine (mg/dL)					
Min. – Max.	0.54 – 0.85	0.49 – 0.86	0.44 – 0.86	2.777	0.068
Mean ± SD.	0.69 ± 0.10	0.67 ± 0.12	0.62 ± 0.12		
Sig. among groups	p_1<0.001*,p_2<0.001*,p_3<0.001*				
eGFR-Cr (mL/min/1.73 m2)					
Min. – Max.	55.60 – 138.70	50.0 – 147.80	67.30 – 160.60	2.619	0.079
Mean ± SD.	94.70 ± 26.50	107.73 ± 31.43	110.64 ± 28.03		
Sig. among groups	p_1<0.001*,p_2<0.001*,p_3<0.001*				
Urinary CyPA (ng/mL)					
Min. – Max.	21.9 – 77.3	29.4 – 49.4	7.1 – 41.3	26.234*	<0.001*
Mean ± SD.	45.54 ± 13.16	40.3 ± 5.58	27.84 ± 8.91		
Sig. among groups	p_1=0.098, p_2<0.001*, p_3<0.001*				

*SD: Standard deviation; F: F for ANOVA examination, Pairwise comparing among every two groups was completed using Post Hoc Examination (Tukey); p: p-value for comparing among studied groups; p_1: p-value for comparing among Group A & Group B; p_2: p-value for comparison among Group A & Group C; p_3: p-value for comparing among Group B & Group C; *: important at p ≤ 0.05; Group A: Microalbuminuric diabetic children; Group B: Normoalbuminuric diabetic children; Group C: Healthy Children.*

Concerning the urinary A/C ratio, those with microalbuminuria had the highest A/C ratio when compared to others. This was important (p<0.001). Regarding serum creatinine levels, we found that despite serum creatinine levels being high among those with microalbuminuria when compared to others, this was statistically insignificant (p=0.068). Similarly, despite serum GFR being high among the healthy control group when compared to others, this was statistically insignificant (p=0.079). Urinary CyPA, showed that those with microalbuminuria had the highest urinary CyPA serum levels when compared to others (p<0.001).

Table 3: Comparing the tested groups according to Urinary CyPA (ng/mL)

Urinary CyPA (ng/mL)	Group A+B (n =60)	Group C (n =30)	t	p
Min. – Max.	21.9 – 77.30	7.10 – 41.30	6.807	<0.001*
Mean ± SD.	42.92 ± 10.37	27.84 ± 8.91		

*SD: Standard deviation, t: Student t-exam, *: significant at p ≤ 0.05*

The relationship between CyPA expression & both onset age of DM and HbA1c serum levels was important (p=0.029, p=0.004) in group B (table 4). Concerning patients with microalbuminuric type 1 DM, the correlation between CyPA urinary excretion and SBP, HbA1c (%), and Urinary albumin to creatinine ratio was statistically important (p = 0.016, p= 0.008, p=0.038) (table 4). Urinary CyPA at cut off value of 49.75 ng /ml, could be a reliable method for the prediction of DN among diabetic patients with type 1 DM. with AUC of 0.632, level of sensitivity of 43.3%, specificity of 83.3%, PPV 72.2%, and NPV 59.5%. This was important (p<0.001) (table 5)

Discussion

The most prevalent endocrine condition in children is type 1 diabetes mellitus which causes several macro- & micro-vascular consequences. Diabetic nephropathy, known as diabetic kidney disease, is one of its most severe microvascular consequences and can potentially proceed to end-stage renal disease years later, necessitating dialysis or kidney transplantation[15]. Cyclophilin A (CyPA), an 18-kDa protein found in urine, has universal properties. It promotes protein folding and protein trafficking and is primarily found in the cytoplasm. Additionally, it functions as a cyclosporine A receptor in cells[16]. Cardiovascular disease, asthma, rheumatoid arthritis, liver injury, and inflammatory illnesses have all been linked to secrete CyPA (sCyPA). It was also found in the plasma of diabetic patients and demonstrated that monocytes secrete it in response to hyperglycemia [6]. In our study, we tried to investigate the relationship between Urinary CyPA & diabetic nephropathy in children with type 1 diabetes mellitus. The study was conducted from May 2021 to December 2021. Sixty studied cases with type 1 DM were recruited consecutively from children & adolescents attending the Diabetic outpatient clinic at Suez Canal University Children's Hospital. Thirty years old & gender-matched children & adolescents were recruited as a control group. This study showed that there was an important relation between the 3 studied groups & age of onset of DM, as the onset was earlier in patients with diabetic nephropathy. Amer

et al[6] and Saif et al[17] described that CypA was a reliable novel indicator for early diagnosis of DN. There was statistically important variation among the 3 studied groups & BMI Z-score. There was statistically insignificant variation between the two studied groups of T1DM and Insulin TDD and SBP.

Table 4: Correlation between Urinary CyPA (ng/mL) and different parameters in the two studied groups of type 1 DM

	Urinary CyPA (ng/mL)			
	Group A (n= 30)		Group B (n= 30)	
	r	P	R	P
Years old	-0.029	0.828	0.158	0.404
Years old at DM onset	-0.182	0.164	0.398	**0.029***
Insulin TDD	-0.062	0.633	-0.002	0.993
BMI Z-score	-0.099	0.173	-0.077	0.686
SBP (mmHg)	0.309	**0.016***	0.152	0.422
HbA1c (%)	0.339	**0.008***	0.506	**0.004***
Total cholesterol (mg/dL)	0.121	0.355	-0.111	0.560
Triglycerides (mg/dL)	0.049	0.709	-0.141	0.456
HDL (mg/dL)	-0.032	0.807	-0.155	0.413
LDL (mg/dL)	-0.020	0.878	-0.083	0.663
Urinary albumin to creatinine ratio	0.268	**0.038***	0.020	0.915
Serum creatinine (mg/dL)	-0.149	0.254	0.0	0.999
eGFR-Cr (mL/min/1.73 m2)	-0.190	0.146	-0.215	0.253

*r: Pearson coefficient; *: important at p ≤ 0.05*

Table 5: Validity of Urinary CyPA (ng/mL) to predict Microalbuminuria in DM type 1 patients (n= 60)

Urinary CyPA (ng/mL)	
AUC	0.632
p	<0.001*
95% CI	0.485 – 0.779
Cut off	>49.75
Sensitivity	43.3
Specificity	83.3
PPV	72.2
NPV	59.5

AUC: Area Under Curve; p-value: Probability value.
CI: Confidence Intervals; NPV: Negative predictive value.
*PPV: Positive predictive value; *: significant at p ≤ 0.05.*

In concordance with our results, Konsouh et al [18] reported a lack of important variation in BMI among 2 groups of T1DM children& adolescents. Maric-Bilkan [19] described that obesity is the main contributing factor to developing DN. In our study, the BMI median was significantly higher between children & adolescents with DM type 1 irrespective of the type of microalbuminuria when compared with years old & gender-matched healthy controls. Katz et al [20] found that diabetic children & adolescents with high BMI were significantly more liable to develop diabetic nephropathy when compared with others with normal BMI. In disa-

greement with our results, Amer et al[6] indicated that BMI was insignificantly related to DN in studied cases with type 1 DM while in agreement with our results, there was insignificant variation among three tested groups & SBP. Concerning HbA1C, our study, showed that its mean level was greater between those with microalbuminuric DM when compared with those with normoalbuminuric one and control group. Similarly, Amer et al. [6] found that diabetic patients with microalbuminuria had a significantly elevated HbA1C when compared with others. In our study, there was an important positive Relationship between Urinary CyPA & HbA1C in diabetic studied cases. This matches what was found by Ebrahim et al[21] who proved that both FBS and HbA1C were high among those with diabetic nephropathy. Concerning lipid profile, children& adolescents with normal albumin excretion had significantly higher levels of HDL when compared with the other two groups. On the other hand, LDL mean serum levels were high among those with microalbuminuria when compared with others. On the other hand, we found that despite mean triglyceride levels being high among those with normoalbuminuric DN, this was statistically insignificant. In agreement with outcomes, Amer et al[6] also found that patients with microalbuminuria had greater serum levels of cholesterol, LDL, and triglycerides. El Dayem et al[22] Lower HDL levels were found in studied cases with microalbuminuria. These perplexing results can be explained by changes in the distribution of lipoprotein subclasses. There was important variation among the 3 studied groups in terms of urinary albumin to creatinine ratio & urinary CyPA in current research. Differences in serum creatinine & eGFRCr among the 3 study groups were statistically insignificant. This was consistent with Amer et al[18] findings which found that there was important variation among studied groups and creatinine, albumin to creatinine ratio & eGFR. Concerning urinary CyPA, it demonstrated a high degree of diagnostic accuracy in detecting the degree of albuminuria in diabetic studied cases. Urinary CyPA was shown to be higher in diabetic studied cases with microalbuminuria than in those with normoalbuminuria in our research. This matches with what was reported by Ebrahim et al[21] who discovered important variation in Urinary CyPA among tested groups of DN & controls. As a result, urinary CyPA can be used as an early sign to recognize DN with high specificity. Urinary CypA detection is very convenient as it is non-invasive. Urinary CypA now appears to be able to detect DN in the silent stage. Also, outcomes were in agreement with Saif et al[17] who discovered that urinary CyPA was greater in studied cases with stage 2 DN than in studied cases with stage 1 DN. Tsai et al[9] described that in Chinese studied cases with diabetic nephropathy, urinary CyPA levels were high with high grades of nephropathy among diabetic patients. In group B, there was an important positive relationship between Urinary CyPA (ng/ml) & both years old of onset of T1DM & HbA1c (percent). Urinary CyPA (ng/ml) had an important positive relationship with SBP, HbA1c, & Urinary albumin to creatinine ratio in group A. Contrary to our study, Tsai et al[9] reported the presence of an important negative correlation between urinary CyPA & eGFR in diabetic nephropathy studied cases (p= 0.013). Also, they found that the relationship of urinary CyPA enlarged by 0.030 ng/ml with every one ml/min decrease in eGFR and they established an equation that illustrated the correlation between Urinary CyPA and eGFR (CyPA=5.270+ GFR*-

0.030). The difference from our results could be because of variations in sample size.

Conclusion

Urinary CyPA was higher in type 1 diabetic children with microalbuminuric DN than diabetics with normoalbuminuric DN. Urinary CyPA correlated positively with the urinary albumin creatinine ratio but negatively with the estimated glomerular filtration rate. Urinary CyPA can be used as an early indicator for DN because we showed high levels of urinary CyPA in diabetic type 1 studied cases with DN before albuminuria appeared.

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