Urinary interleukin-6 and tumour necrosis factor-alpha as early markers for diabetic nephropathy in children and adolescents with type 1 diabetes

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Sri Lanka Journal of Child Health, 2020; **49**(3): 269-275

Abstract

Background: Diabetic nephropathy (DN) is the main cause of morbidity and mortality in young adults with type 1 diabetes mellitus (T1DM). The accuracy of albuminuria has been frequently questioned as a predictor and prognosticator of renal injury. Therefore, new urinary markers were investigated for the early detection of DN.

Objectives: To assess the potential value of urinary interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) as early markers for detection of DN in children and adolescents with T1DM.

Method: This case-control study was conducted on 50 children and adolescents with T1DM, aged ≤ 18 years. Patients were classified according to their albumin/creatinine ratio (ACR) into two groups, normo-albuminuric and albuminuric, together with 25 age and sex-matched healthy controls. ACR, urinary IL-6 and TNF-α levels were assayed for patients and controls.

Results: Urinary interleukin-6/creatinine (IL-6/Cr) ratio of albuminuric patients was significantly higher compared to normo-albuminuric patients or controls. Urinary IL-6/Cr ratio ≥0.24pg/mg was a significant cutoff value to detect albuminuria with 72% sensitivity and 52% specificity (p=0.04). We did not observe different urinary TNF-α/creatinine (TNF-α/Cr) ratio in the studied groups, or between albuminuric and normo-albuminuric patients or controls.

Conclusions: Urinary IL-6/Cr ratio seems to be a promising new marker for early detection of DN in patients with T1DM, while urinary TNF-α /Cr ratio showed no significant difference between diabetic patients and controls.

http://dx.doi.org/10.4038/sljch.v49i3.9146

(Key words: Type 1 diabetes mellitus, diabetic nephropathy, urinary interleukin-6, urinary tumour necrosis factor-alpha, albuminuria)

Introduction

Complications of diabetes mellitus (DM) include diabetic nephropathy (DN), retinopathy, and cardiovascular disease[1]. DN is the most serious microvascular complication and is the leading cause of chronic kidney disease, which frequently leads to end stage renal disease[2]. Microalbuminuria has been identified in clinical practice as the earliest predictor of DN but a high percentage of renal impairment occurs before the onset of microalbuminuria or even in the non-albuminuric state. Thus, urinary albumin levels may merely reflect an initial reversible stage of renal damage[3]. Therefore, detection of new predictive markers to use with microalbuminuria during the early stages of diabetic kidney disease would provide a chance of preventing or delaying the onset of irreversible consequences through prohibitive therapeutic interventions[4]. Hyperglycaemia can promote the expression of some pro-inflammatory cytokines, like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), which lead to a chronic subclinical inflammatory state in DM[5]. IL-6 is a pleiotropic cytokine and its serum level was found to have a significant correlation with the severity of diabetic glomerulopathy, kidney hypertrophy, and albumin excretion suggesting its role in the pathogenesis of DN[6]. TNF-α is a major contributor to inflammation, apoptosis and extracellular matrix accumulation in glomerular and tubular regions leading to alteration of glomerular filtration, tubular permeability and reabsorption[3].

Objectives

To study urinary IL-6 and TNF-α as early markers for detecting DN in children and adolescents having type 1 diabetes mellitus (T1DM).

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(Received on 22 October 2019: Accepted after revision on 20 December 2019)
The authors declare that there are no conflicts of interest
Personal funding was used for the project.

Method

A case-control study was conducted at the Diabetes, Endocrine & Metabolism Paediatric Unit (DEMPU), Cairo University Children's Hospital in 50 children and adolescents with T1DM >5 years. They were classified based on their albumin/ creatinine ratio (ACR) into 2 groups. Group I (n=25) was normo-albuminuric (ACR<30mg/g) and Group II (n=25) was albuminuric (ACR 30-300mg/g). Twenty five healthy age & sex-matched children and adolescents acted as controls. Patients with cardiac, hepatic or renal disease other than DN were excluded from study.

All patients had a complete history taken with special emphasis on age of onset and duration of diabetes, insulin therapy and any history suggestive of renal complications, including albuminuria & hypertension. A thorough physical examination was performed on all patients, laying stress on anthropometric measurements in terms of standing height, height standard deviation score (SDS), weight and weight SDS, body mass index (BMI), and BMI SDS for age and sex using software program Growth Vision 2.0 provided by Novo-Nordisk, Denmark. Further, blood pressure (BP) was measured and plotted on BP tables according to age and height percentile for boys and girls[7]. Puberty was assessed using the Tanner staging[8].

Serum creatinine (sCr) was determined and estimated glomerular filtration rate (eGFR) calculated by Schwartz formula: eGFR = K x Height (cm)/sCr (mg/dL). K was equal to 0.55 in 5-13 year old children and adolescent females, whilst it was 0.7 in adolescent males[9]. Lipid profile, including total cholesterol, serum high density lipoprotein-cholesterol (HDL-c), and triglyceride concentration, were determined enzymatically using commercially available kits on an auto-analyser (Olympus AU 480, USA). Low density lipoprotein-cholesterol (LDL-c) was estimated using Friedewald Formula[10]. The mean of glycosylated haemoglobin (HbA1c) values in the last year were recorded. Mean values for fasting and postprandial plasma glucose readings were calculated from the patients' notes in the previous month.

In addition, first-morning urine samples were collected. Complete urine analysis was performed and albuminuria was assessed in the absence of confounders namely urinary tract infections, exercise, and menstrual bleeding. ACR was determined by the immune-nephelometric method on Prospec Siemens, Siemens Healthcare Diagnostic Inc. Newark, DE 19714 U.S.A[11]. Albuminuria was confirmed by two ACR > 30 mg/g on 2 separate occasions. Urinary IL-6 and TNF-α levels were assayed for patients and controls using a commercial enzyme-linked immunosorbent assay (ELISA) kits (Human IL-6 ELISA Kit. Catalog No: E0079h; Human TNF-α ELISA Kit. Catalog No: E0113h) in accord with the manufacturer's instructions. Urinary levels of cytokines were corrected by urinary creatinine (Cr) concentration and expressed as urinary IL-6/Cr ratio and TNF-α/Cr ratio.

Statistical analysis was done using the Statistical Package for the Social Sciences version 22. Data normally distributed were expressed as mean ± SD and compared by ANOVA and Student *t* -test. Data not normally distributed were expressed as median and interquartile range (IQR). Shapiro-Wilk test was utilized to test if continuous variables were normally distributed. Comparison of the ratios between the studied groups was done using Kruskal Wallis test and Mann Whitney test as posthoc multiple 2-group comparisons after applying Bonferroni adjustment of multiple comparisons. Frequency differences were assessed using Chi-square test. Correlation between variables was performed utilising Spearman rank correlation equation. Multivariate linear regression analysis was utilised to test for the independent predictors of studied ratios in each of the diseased groups. Receiver operator characteristic (ROC) curves were constructed to assess reliability of urinary IL6/Cr ratio and TNF-α/Cr ratio in early detection of albuminuria. The area under the curve (AUC) was considered significant if >0.6 with a significant p-value. Two-sided p-value <0.05 was considered statistically significant.

Ethical issues: Informed consent was obtained from parents of all patients before inclusion in the study. All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the ethical committee of the Pediatric Department, Faculty of Medicine, Cairo University, and received as well, the approval of the Research Ethics Committee, Faculty of Medicine, Cairo University, date of approval: May 8, 2016, approval no. I-020315 and I-030315) and with the Helsinki Declaration of 2013.

Results

Of the 50 children and adolescents with T1DM, 26 were female and 24 were male. Group I had 10 girls and 15 boys with a mean age of 13.04 ± 2.76 years. Group II had 16 girls and 9 boys with a mean age of 13.64 ±3.28 years. Among the 25 healthy, age and sex-matched controls, 13 were female and 12 were male. Their ages ranged from 8 to 18 years with a mean age of 13±2.75 years. Apart from the significantly higher systolic and diastolic blood pressures in albuminuric patients, no significant differences were noted regarding clinical and demographic data between both patient groups. Regarding the laboratory findings, there was only a significantly higher mean fasting plasma glucose level in the albuminuric patients (Table 1).

Table 1: ***Clinical, demographic and laboratory data of normo-albuminuric and albuminuric groups***

Parameters	Normo-albuminuric group *(n=25)*	Albuminuric group *(n=25)*	*p*
Diabetes duration (years) Mean± SD	7.32± 2.14	7.88± 2.99	0.450
Age of onset (years) Median (IQR)	6 (3)	5 (3)	0.761
Insulin dose (IU/kg/day) Mean± SD	1.36± 0.49	1.28± 0.46	0.554
Height (cm) Mean± SD	143.02±12.18	145.21±14.46	0.565
Height standard deviation score Median (IQR)	-1.5 (-1.7)	-1.13 (-2.02)	0.62
Weight (kg) Mean± SD	40.12±10.94	43.7±13.12	0.300
Weight standard deviation score Median (IQR)	-0.5 (-0.8)	-0.2 (-0.59)	0.567
Body mass index (kg/m^2) Mean± SD	19.28±3.02	20.26±2.99	0.256
Body mass index standard deviation score Median (IQR)	0.4 (0.61)	0.5 (0.91)	0.58
Systolic blood pressure (mmHg) Mean± SD	107.96±10.22	116.2±15.56	0.032 *
Diastolic blood pressure (mmHg) Mean± SD	70.4±8.53	74.52±18.38	0.029 *
Mean fasting plasma glucose (mg/dl) Mean± SD	141.5 (60.7)	165.3 (66.9)	0.02 *
Mean postprandial plasma glucose (mg/dl) Median (IQR)	220 (75)	253.4 (48.5)	0.097
Glycosylated haemoglobin (%) Mean± SD	8.75±1.62	9.38±2.26	0.266
Serum creatinine (mg/dl) Mean± SD	0.69±0.21	0.62±0.13	0.128
Glomerular filtration rate (ml/min/1.73m^2) Mean± SD	119.51 (45.6)	141.17 (39.7)	0.138
Cholesterol (mg/dl) Median (IQR)	157 (27)	170 (56)	0.554
Triglycerides (mg/dl) Median (IQR)	90 (52)	66 (55)	0.281
High density lipoprotein-cholesterol (mg/dl) Mean± SD	46.35±10.14	51.00±8.38	0.083
Low density lipoprotein-cholesterol (mg/dl) Median (IQR)	90 (37.7)	106 (44)	0.467
Albumin/creatinine ratio (mg/g) Median (IQR)	10.4 (7.8)	57.4 (49.5)	<0.001*

*For Mean± SD 'Student t-test' was used as test of significance. For Median (IQR) 'Mann Whitney test' was used. *p<0.05 IQR—interquartile range; IU—international unit; SD—standard deviation*

Table 2 shows a significant difference between urinary IL-6/Cr ratio among the studied groups. The albuminuric patients had significantly higher urinary IL-6/Cr ratio when compared to normo-albuminuric patients or controls. However, we did not observe varying TNF-α/Cr ratio in the studied groups, or between albuminuric and normo-albuminuric patients or controls. Similarly, there was no significant difference in urinary IL-6 and TNF-α levels between the studied groups. Nonetheless, there was a significant correlation between urinary IL-6 and TNF-α among the studied patients (p= 0.018) and controls (p= 0.004).

Table 2: ***Urinary IL-6, TNF-α and their Cr ratio in the studied groups***

Urinary cytokines	Normo-albuminuric group (n=25)	Albuminuric group (n=25)	Controls n=25	p
	Median (IQR)	Median (IQR)	Median (IQR)	
IL-6 (pg/ml)	25 (5)	25 (10)	20 (20)	0.22
IL-6/Cr ratio	0.23 (0.5)	0.37 (7.91)	0.2 (0.57)	0.018*
(pg/mg)				0.23[a]
				0.01*[b]
				0.04*[c]
TNF-α (pg/ml)	20 (10)	15 (10)	20 (15)	0.15
TNF-α/Cr ratio	0.75 (0.4)	0.8 (0.5)	0.83 (0.55)	0.7
(pg/mg)				0.56[a]
				0.4[b]
				0.89[c]

*Not normally distributed data were expressed as Median (IQR) and compared by the Kruskal–Wallis or Mann–Whitney U test. Cr—creatinine, IL-6—interleukin-6, IQR—interquartile range; TNF-α—tumor necrosis factor alpha * Significant a. Normo-albuminuric group versus Control group b. Albuminuric group versus Control group c. Normo-albuminuric group versus Albuminuric group*

Urinary IL-6/Cr ratio was negatively correlated with the duration of diabetes (r= -0.43, p=0.029), and positively correlated with the urinary TNF-α level (r=0.54, p=0.005) in albuminuric patients, and it also had a negative correlation with fasting plasma glucose (r= -0.62, p=0.001) in normo-albuminuric patients (Table 3). A multiple regression analysis was performed between urinary IL-6/Cr ratio and diabetes duration as well as urinary TNF-α level, but no statistically significant association was found.

Urinary TNF-α/Cr ratio had a positive correlation with duration of diabetes (r=0.48, p=0.01) and systolic blood pressure (r= 0.4, p=0.04) in normo-albuminuric patients, and also with LDL-c (r=0.41, p=0.03) in albuminuric patients while it had a negative one with urinary IL-6 level in both groups (r= -0.49, p=0.01) (r= -0.68, p=0.00) (Table 4). A multiple regression analysis was performed between urinary TNF-α/Cr ratio and diabetes duration, systolic blood pressure (SBP), LDL-c as well as urinary IL-6 level, but no statistically significant correlation was reported. Similarly, there was no significant correlation between either urinary IL-6 or TNF-α among studied patient groups as regards any of the clinical or laboratory data (Tables 3 and 4).

Table 3: ***Correlation between urinary IL-6 and IL-6/Cr ratio with the patients' clinical data and laboratory findings***

Parameters	Urinary IL-6 (pg/ml)				Urinary IL-6/Cr ratio (pg/mg)			
	Normo-albuminuric group n=25		Albuminuric group n=25		Normo-albuminuric group n=25		Albuminuric group n=25	
	r	*p*	r	*p*	r	*p*	r	*p*
Diabetes duration (years)	-0.395	0.051	-0.269	0.193	-0.12	0.55	-0.43	0.02*
Age of onset (years)	-0.072	0.732	0.044	0.836	-0.09	0.64	0.21	0.3
Insulin dose (IU/kg/day)	-0.080	0.704	0.070	0.740	-0.2	0.33	0.1	0.6
Height standard deviation score	0.354	0.053	0.056	0.789	0.15	0.45	-0.08	0.67
Weight standard deviation score	0.215	0.302	0.121	0.565	0.28	0.17	-0.07	0.72
BMI standard deviation score	0.136	0.517	0.162	0.439	0.25	0.22	0.04	0.83
Systolic blood pressure (mmHg)	-0.332	0.105	-0.078	0.711	0.007	0.97	-0.12	0.56
Diastolic blood pressure (mmHg)	-0.187	0.370	0.165	0.430	0.13	0.51	0.02	0.9
Fasting plasma glucose (mg/dl)	-0.228	0.274	0.019	0.927	-0.62	0.001*	0.09	0.65
Glycosylated haemoglobin (%)	-0.240	0.249	-0.309	0.133	0.19	0.34	-0.26	0.2
Serum Creatinine (mg/dl)	-0.275	0.184	-0.043	0.840	0.001	0.99	-0.004	0.98
GFR (ml/min/1.73m^2)	0.041	0.846	0.070	0.741	-0.1	0.62	-0.06	0.76
Cholesterol (mg/dl)	-0.192	0.357	-0.199	0.341	-0.2	0.31	0.02	0.89
Triglycerides (mg/dl)	-0.082	0.696	0.007	0.974	-0.06	0.76	0.22	0.28
HDL-c (mg/dl)	0.262	0.206	-0.193	0.356	0.15	0.44	-0.21	0.31
LDL-c (mg/dl)	-0.135	0.520	-0.096	0.649	-0.11	0.59	0.18	0.37
ACR (mg/gm)	-0.162	0.439	0.216	0.299	0.32	0.11	0.16	0.43
TNF-α (pg/ml)	-	-	-	-	0.25	0.22	0.54	0.005*

*r — Spearman coefficient, p value was significant at p < 0.05. * Significant*
ACR—albumin/creatinine ratio; BMI— body mass index; GFR—glomerular filtration rate; High density lipoprotein-cholesterol; IU—international unit; LDL-c—Low density lipoprotein-cholesterol; SDS—standard deviation score; TNF-α—tumor necrosis factor alpha

Table 4: ***Correlation between urinary TNF-α and TNF-α/Cr ratio with the patients' clinical data and laboratory findings***

Parameters	Urinary TNF-α (pg/ml)				Urinary TNF-α/Cr ratio (pg/mg)			
	Normo-albuminuric group (n=25)		Albuminuric group (n=25)		Normo-albuminuric group (n=25)		Albuminuric group (n=25)	
	r	*p*	r	*p*	r	*p*	r	*p*
Diabetes duration (years)	0.258	0.213	-0.207	0.321	0.48	0.01*	0.05	0.8
Age of onset (years)	-0.034	0.870	0.277	0.180	-0.09	0.66	0.29	0.14
Insulin dose (IU/kg/day)	-0.273	0.187	0.155	0.458	0.01	0.95	0.003	0.99
Height standard deviation score	-0.393	0.052	-0.125	0.55	-0.56	0.003*	-0.004	0.98
Weight standard deviation score	-0.321	0.118	0.024	0.909	-0.46	0.01*	-0.06	0.74
Body mass index SDS	-0.122	0.563	-0.006	0.979	-0.23	0.26	-0.26	0.2
Systolic blood pressure (mmHg)	0.287	0.164	0.307	0.136	0.4	0.04	0.29	0.15
Diastolic blood pressure (mmHg)	0.025	0.906	0.352	0.085	0.09	0.66	0.05	0.8
Fasting plasma glucose (mg/dl)	-0.262	0.206	0.212	0.310	-0.23	0.26	0.11	0.57
Glycosylated hemoglobin (%)	0.064	0.762	-0.297	0.149	0.07	0.71	0.15	0.47
Serum creatinine (mg/dl)	0.236	0.256	0.078	0.710	0.36	0.07	0.12	0.56
GFR (ml/min/1.73m^2)	-0.275	0.183	0.042	0.841	-0.23	0.25	-0.02	0.9
Cholesterol (mg/dl)	-0.237	0.254	0.236	0.255	-0.16	0.44	0.37	0.06
Triglycerides (mg/dl)	0.285	0.168	0.022	0.918	0.18	0.38	-0.04	0.84
HDL-c (mg/dl)	-0.268	0.195	-0.074	0.727	-0.36	0.07	0.21	0.29
LDL-c (mg/dl)	-0.195	0.349	0.387	0.056	-0.13	0.5	0.41	0.03*
Albumin/creatinine ratio (mg/g)	0.251	0.226	-0.001	0.997	0.33	0.1	-0.31	0.12
Interleukin-6 (pg/ml)	-	-	-	-	-0.49	0.01*	-0.68	0.00*

*r — Spearman coefficient, p value was significant at p < 0.05. * Significant*
GFR—glomerular filtration rate; HDL-c—High density lipoprotein-cholesterol; IU—international unit; LDL-c—Low density lipoprotein-cholesterol; SDS—standard deviation score; TNF-α—tumor necrosis factor alpha;

Urinary IL-6/Cr ratio ≥0.24pg/mg was a significant cutoff value to detect albuminuria with 72% sensitivity and 52% specificity (p= 0.04) which was documented by ROC curve (AUC =0.666) indicating that, urinary IL-6/Cr ratio could be a potential marker for early diagnosis of DN (Figure 1).

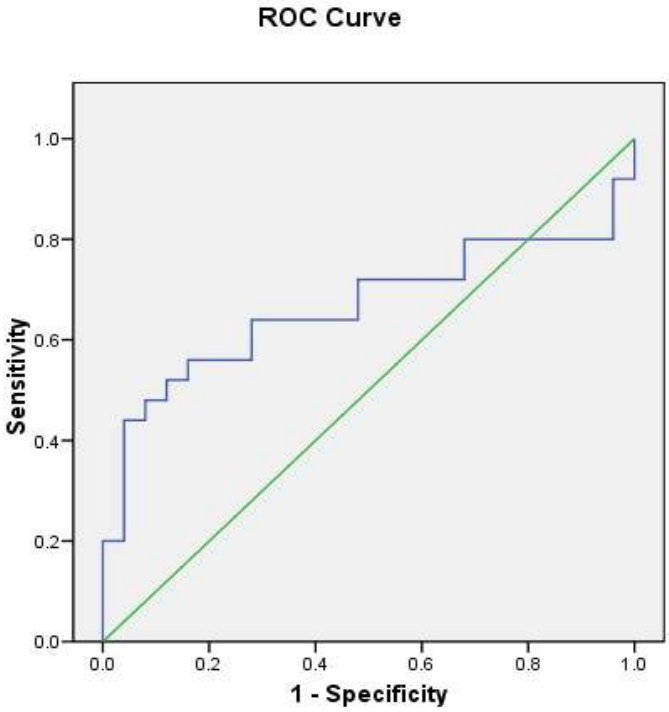

Figure 1 ***ROC curve of urinary IL-6/ Cr ratio cutoff value to detect albuminuria***

IL-6/ Cr ratio ≥ 0.24 pg/mg showed a significant cutoff value to detect albuminuria with 72% sensitivity and 52% specificity (p= 0.04).*

IL-6/Cr ratio— interleukin-6/creatinine ratio; Area under the curve (AUC) = 0.666.

** p value was significant (p < 0.05)*

Discussion

As the mechanisms responsible for development of renal injury are still unclear, inflammation had a critical role in the development of early injury in preclinical DN[12]. Therefore, we were encouraged to assess urinary markers of inflammation which could predict early DN in children and adolescents with T1DM. In our study, elevated urinary IL-6/Cr ratio was reported with the worsening of albuminuria, since higher values were noted in the albuminuric in comparison with normo-albuminuric patients or controls. In addition, urinary IL-6/Cr ratio ≥0.24pg/mg was a significant cutoff value to detect albuminuria with 72% sensitivity and 52% specificity (p= 0.04). We did not note significant differences in TNF-α/Cr ratio between the studied groups, or between albuminuric and normo-albuminuric patients or controls. These findings support the inflammatory role of urinary IL-6 and its subsequent increase in DN indicating that urinary IL-6/Cr ratio could be a potential marker of early DN. This is in accordance with Pestana *et al*[12] who reported elevated urinary IL-6/Cr ratio in patients with micro- and macro-albuminuria in comparison with normo-albuminuric patients and controls. Further, Vaidya *et al*[13] noted that micro-albuminuric patients had significantly higher urinary IL-6 than normo-albuminuric patients. Furthermore, Wolkow *et al*[14] reported that urinary IL-6 concentrations were comparable in the patients with normal renal functions either normo-albuminuric or micro-albuminuric, whereas they were significantly higher in the micro-albuminuric group with a progressive decline of renal functions. Nevertheless, urinary TNF-α was not detectable in most patients, and they concluded that urinary IL-6 was associated with declining renal function, but not linked to microalbuminuria. This was contrary to Klimontov *et al*[15] and Pestana *et al*[12] who found that urinary TNF-α level in patients with micro- and macro-albuminuria were significantly increased compared to controls.

On univariate analysis, there was a significant association between urinary IL-6/Cr ratio with diabetes duration, fasting plasma glucose (FPG) and urinary TNF-α level. Further, we observed a significant correlation between the urinary TNF-α/Cr ratio with duration of diabetes, SBP, low density lipoprotein-cholesterol, and urinary IL-6 level. However, no significant association was documented when the markers were entered into the multiple regression model. This lack of significant correlation in our study between urinary cytokines and all the studied clinical and laboratory data among the patient groups might demonstrate the complex nature of the disease and reflect the persistent inflammatory milieu in T1DM whether of long or short duration. This is consistent with

Pestana *et al*[12] who studied urinary IL-6/Cr ratio and TNF-α/Cr ratio in T1DM patients and found that urinary IL-6/Cr ratio was not correlated with sCr, albuminuria, eGFR, FPG, and HbA1c. However, urinary TNF-α/Cr ratio was reported to have been positively correlated with sCr and albuminuria, albeit negatively correlated with eGFR. Vaidya *et al*[13] reported that microalbuminuria progression and regression were unrelated to the urinary IL-6 level.

We recorded significantly higher SBP and DBP measurements in patients with albuminuria compared to normo-albuminuric patients. This was in concordance with Amritanshu *et al*[16] and Ahmed[17] who reported that in patients with microalbuminuria, both SBP and DBP values were significantly higher in comparison with those having normo-albuminuria. On the other hand, Suh *et al*[18] observed that SBP and DBP did not differ between the normo-albuminuric and micro-albuminuric groups. We also found albuminuric patients had significantly higher FPG levels in comparison to normo-albuminuric patients. This was endorsed by Shelbaya *et al*[19] and Pestana *et al*[12] while we did not report any statistically significant difference as regards HbA1c. This was similar to Shelbaya *et al*[19] and Suh *et al*[18]. Nevertheless, this was in contrast to the findings of Ahmed[17] and Pestana *et al*[12] whose studies revealed statistically higher HbA1c levels among the micro-albuminuric group. These attractive findings support that HbA1c values do not exactly consider the factual state of glycaemic control in some circumstances with rapid alterations in glycaemic control and anaemia and patients with variant haemoglobin[20].

In addition, no statistically significant difference was observed between our patient groups as regards sCr and eGFR, which was in agreement with Ahmed[17] and Suh *et al*[18]. These findings support that sCr in diabetic patients does not indicate the early signs of diminished renal functions[21]. However, Pestana *et al*[12] revealed that the albuminuric patients had significantly higher sCr and lower eGFR than the normo-albuminuric patients. The fact that this was a case-control study with relatively few subjects is a limitation. More extensive studies of a longitudinal nature are required to confirm the association between urinary cytokines such as IL-6 and TNF-α with the renal pathophysiology in children with T1DM.

Conclusions

Urinary IL-6/Cr ratio was elevated with worsening of the nephropathy since higher values were observed in the albuminuric patients when compared to normo-albuminuric patients and controls. Urinary IL-6/Cr ratio ≥0.24pg/mg was a significant cutoff value to detect albuminuria with 72% sensitivity and 52% specificity (p=0.04). Urinary IL-6/Cr ratio seems to be a promising new marker for early detection of DN in patients with T1DM, while urinary TNF-α /Cr ratio showed no significant difference between diabetic patients and controls.

Acknowledgments

We would like to express our sincere thanks to all children and their parents who participated in this work. We would like to express our appreciation to our colleagues and nurses at Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU) who facilitated this work.

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