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ORIGINAL ARTICLE

Urinary markers in the early stage of nephropathy in patients with childhood-onset type 1 diabetes

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Abstract
Background The aim of this study was to evaluate the association of a urinary tubular marker, liver-type fatty acid binding protein (L-FABP) and an inflammatory marker, serum/urinary YKL-40, with albuminuria in patients with childhood-onset type 1 diabetes (T1D).
Methods Twenty-nine patients with childhood-onset T1D and 32 controls were enrolled. Serum and urinary concentrations of YKL-40 and urinary concentrations of L-FABP were measured.
Results The serum levels of YKL-40 were not significantly different between the control group and the patient groups. However, the levels of urinary YKL-40/creatinine (Cr) were higher in the patients, even those with normoalbuminuria than in the controls ($p<0.001$). The levels of urinary L-FABP/Cr were not different between the control group and the patient groups. However, the level of urinary L-FABP/Cr in the microalbuminuria group was higher than that in the normoalbuminuria group ($p=0.03$). There were no associations between the levels of urinary albumin-to-creatinine ratio and urinary L-FABP/Cr or YKL-40/Cr. However, the urinary L-FABP/Cr level was significantly correlated with the hemoglobin A1C level ($p=0.005$) and the urinary YKL-40/Cr level ($p=0.043$).
Conclusions Urinary L-FABP/Cr and YKL-40/Cr may reflect renal injury in early stages of nephropathy in patients with childhood-onset T1D, even in the normoalbuminuric state.

Keywords Liver-type fatty acid binding protein · YKL-40 · Albuminuria · Biomarkers · Diabetic nephropathy · Children

Introduction

Diabetic nephropathy (DN) is one of the most important complications in patients with diabetes, having an adverse effect on morbidity and mortality. At present, albuminuria is widely used as an early clinical marker for the detection of DN. However, several studies have questioned the reliability of this measure as an indicator of renal injury and as a predictor of its progression, because significant pathological changes or declines in the estimated glomerular filtration rate (eGFR) can occur even in patients with normoalbuminuria (NA) [1–3].

Liver-type fatty acid binding protein (L-FABP) is a protein expressed in the proximal tubule of the kidney and is involved in fatty acid metabolism [4, 5]. In patients with renal tubulointerstitial injury, renal gene expression for *FABP1* (fatty acid binding protein, liver) is upregulated and the urinary excretion of L-FABP is increased [6, 7]. Urinary L-FABP has been shown to be a noninvasive and suitable marker for renal injury, not only for the early prediction of acute kidney injury but also for the progression of chronic kidney diseases [5]. Several studies on patients with adult type 1 or 2 diabetes have demonstrated that urinary L-FABP reflects tubulointerstitial injury even in patients with normoalbuminuria, and its level is increased in proportion to DN severity [8, 9]. Moreover, the concentrations of urinary L-FABP are decreased with renoprotective therapies such as angiotensin-converting

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enzyme inhibitors (ACEI) [10]. However, the association of urinary L-FABP in patients with childhood-onset type 1 diabetes (T1D) has rarely been evaluated.

YKL-40, its abbreviation is derived from the one letter code for the first three N-terminal amino acids, tyrosine (Y), lysine (K), and leucine (L) and the apparent molecular weight of YKL-40 (40 kD), is a member of the mammalian chitinase-like protein family and is also called cartilage glycoprotein-39 [11, 12]. It is expressed in a variety of cells including macrophage, neutrophils and endothelial cells, and its expression is regulated by various cytokines and hormones [13]. YKL-40 has been implicated in diverse biological processes such as extracellular matrix remodeling, fibrosis, angiogenesis and inflammation [14], and elevated circulating or local tissue levels of YKL-40 have been observed in patients with cancers, cardiovascular diseases, infectious diseases, and some autoimmune diseases [15–19]. Recently, several studies reported the associations of YKL-40 and renal diseases. Plasma YKL-40 levels were elevated in patients with adult type 1 or 2 diabetes compared with normal controls and there was a significant positive association between YKL-40 levels and urinary albumin excretion [20, 21]. Urinary YKL-40 levels were elevated in recipients of kidney transplantation with delayed graft function than those with slow or immediate graft function [22], and similar results were found in hospitalized patients with progressive acute kidney injury [23]. However, to date, little information has been obtained about YKL-40 levels in children with diabetes.

Here, we evaluated associations between urinary L-FABP and serum/urinary YKL-40 levels with the levels of albuminuria in patients with childhood-onset T1D.

Methods

Subjects

This was a case–control and cross-sectional study performed in type 1 diabetic patients and healthy controls at the Department of Pediatrics, the Catholic University of Korea, Yeouido, Bucheon and Seoul St. Mary's Hospitals between April 2013 and September 2014. A total of 29 patients with childhood-onset T1D and 32 healthy children were enrolled.

All patients had been diagnosed with T1D under the age of 18 and had an estimated glomerular filtration rate (eGFR) ≥60 ml/min/1.73 m^2. In our institutes, screening for microalbuminuria in type 1 diabetic patients is undertaken every 6 months or more frequently if microalbuminuria exists, by the spot urine albumin-to-creatinine ratio (ACR), and the patients having persistent microalbuminuria for more than 6 months are treated with ACEI after consulting with nephrologists. However, the patients in the present study had no history of medication including ACEI other than insulin. In addition, patients who had other renal diseases or urological problems, active or chronic persistent infections or inflammatory disorders, neoplastic disorders, uncontrolled thyroid disorders, or severe liver dysfunction were excluded. The patients with diabetes enrolled in this study were divided into two groups according to their albuminuria status at the time of enrollment, based on the ACR. Twenty patients had normoalbuminuria (NA) (<30 mg/g creatinine) and nine had microalbuminuria (MA) (30–299 mg/g creatinine). The control group consisted of healthy children without any infection or history of chronic diseases including diabetes and renal disorders.

Measurements

Random spot urine and blood samples were obtained from subjects during their visit to the clinic. Medical histories and anthropometric measurements including systolic and diastolic pressures, height, weight and BMI (body mass index, calculated as weight (kg)/height (m^2)) were also recorded on the same day. BMI and blood pressure scores were expressed in *z* scores, according to the standards assessed by the 2007 Korea National Growth Charts and blood pressure normogram in Korean children and adolescents, respectively [24, 25]. Serum and urine samples were immediately centrifuged at 4 °C for 15 min at 3000 × *g* within 30 min of collection and were stored at −80 °C until final analyses were carried out. The eGFR was calculated using the revised Schwartz equation: 0.413 × height (in cm)/serum creatinine (mg/dl).

Serum levels of YKL-40 and urinary levels of YKL-40 and L-FABP in spot urine samples were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits. Human chitinase 3-like 1 kits for YKL-40 and Human L-FABP kits were purchased from R&D Systems (Minneapolis, MN, USA). All the procedures we used followed the manufacturer's instructions. The respective inter- and intra-assay precisions were 4.3–4.7 % and 5.3–6.9 % for YKL-40 and 3.5–7.9 % and 9.6–11.9 % for L-FABP. Urinary levels of YKL-40 and L-FABP were normalized to the urinary creatinine concentration. All samples were run in duplicate and were within the range of the standard curve. The detection limits were 3.55 pg/ml for YKL-40 and 3.0 ng/ml for L-FABP. Urinary YKL-40 levels for some control subjects were under the detectable range. Published data for healthy individuals also showed that urinary YKL-40 is typically undetectable or less than 0.2 ng/ml [22, 23, 26]. Accordingly, values below the detection limit (3.55 pg/ml for YKL-40) were approximated to 1.775 pg/ml (the mean value between 0 and the lower limit of detection).

Semiquantitative scoring for the concentrations of urinary biomarkers (urinary YKL-40 and urinary L-FABP)

To evaluate the effect of two urinary markers combined together for reflecting renal injury, we performed further analyses by scoring the levels of urinary YKL-40/Cr and urinary L-FABP/Cr. The levels of these urinary markers were scored as a three-point scale as follows. A score of 0 was assigned when urinary YKL-40/Cr or L-FABP/Cr levels were less than the upper quartile value of control group. A score of 1 indicated these levels were greater than or equal to the upper quartile value of control group but less than the upper quartile value of diabetic patient groups (normo and microalbuminuria groups), and a score of 2 indicated that these levels were greater than or equal to the upper quartile value of patient groups. The sum of scores for each subject was calculated by adding the score of urinary YKL-40/Cr on the score of urinary L-FABP/Cr. The score for each urinary marker and the sum of scores for two urinary markers were compared in three groups.

Statistical analyses

Data are presented as the mean±standard deviation (SD) for normally distributed values and the median and interquartile range for nonparametric values. Categorical variables are expressed as frequencies and proportions. Differences between the three groups were analyzed by analysis of variance (ANOVA), followed by Bonferroni's test for normally distributed values and by the Kruskal–Wallis test for nonparametric values. Differences between two groups were analyzed using unpaired Student's *t* tests (parametric distributions) or the Mann–Whitney *U* test (nonparametric distribution). Comparisons of scores between groups were evaluated by the Fisher's exact test or linear by linear association. The associations between the serum or urinary YKL-40/Cr and urinary L-FABP/Cr levels, and the clinical parameters were analyzed using Spearman's correlation coefficient *r*. Statistical analyses were performed using IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Significance was defined as $p<0.05$.

Results

Baseline characteristics

The baseline characteristics and laboratory data of the study subjects are listed in Table 1. The subjects in the type 1 diabetes NA and MA patient groups were well matched with those in the control group in regard to sex, body mass index (BMI), systolic and diastolic blood pressure, and C-reactive protein (CRP) levels. However, subjects in the patient groups were older than subjects in the control group (11.91±3.61 years for the control group vs. 16.52±4.11 years for the patient groups). Subjects in the control and patient groups demonstrated a well-preserved renal function, with an eGFR of 106.66±20.73 ml/min/1.73 m^2 for the control group and 92.09±18.72 ml/min/1.73 m^2 for the patient groups. However, the eGFR levels in the patient groups were significantly lower than those in the control group ($p=0.006$), although the levels did not differ significantly between the normoalbuminuria and microalbuminuria groups. The other clinical and laboratory characteristics including sex, age (both at the time of taking blood or urine samples and when being diagnosed with diabetes), BMI, blood pressure, the duration of diabetes, hemoglobin (Hb) type A1C levels and lipid profiles did not differ between the normoalbuminuria and microalbuminuria groups, except for the level of total cholesterol. The total cholesterol levels were significantly higher in the microalbuminuria group than in the normoalbuminuria group ($p=0.02$).

Differences in serum or urinary markers between each group

Table 2 shows the differences in serum and urinary marker levels between the control, NA, and MA groups. The serum levels of YKL-40 were not significantly different among the three groups. However, the levels of urinary YKL-40/Cr were significantly higher in the patient groups than in the control group ($p<0.001$). The levels were also higher in the NA group, as well as in the MA group than in the control group. However, the levels were not significantly different between the NA and MA groups. The levels of urinary L-FABP/Cr were not significantly different between the patient groups and the control group, though the levels of urinary L-FABP/Cr in the NA group showed a tendency to be higher than those in the control group. However, the levels of urinary L-FABP/Cr in the MA group were significantly higher in comparison to both groups, the control group ($p=0.005$) and the NA group ($p=0.03$).

Differences in the scores for urinary markers between each group

As shown in Table 3, the scores for urinary YKL-40/Cr were significantly higher in the patient groups, even higher in the NA group as well as in the MA group than in the control group, though those did not differ between the MA and NA groups. The scores for urinary L-FABP/Cr were also higher in the patients groups and in the MA group than in the control group. However, the scores did not differ between the NA group and the control group, or between the MA and NA groups. The sum of two scores was significantly higher in the patient groups, even higher in the NA group as well as in the MA group than in the control group, though those did not differ the MA and NA groups.

Table 1 Baseline characteristics and laboratory data of the study subjects

	Control	Patients with type 1 diabetes		*p* value
		NA	MA	
Number	32	20	9	
Sex, male/female	13/19	9/11	3/6	0.838
Age at sampling, years	11.91±3.61	16.35±3.51	16.89±5.44	<0.001*, 0.750**
Age at diagnosing diabetes		10.40±3.42	12.44±2.46	0.12
BMI, *z* score	0.10 (−0.68–0.75)	0.77 (−0.59–1.57)	0.02 (−0.50–0.31)	0.49
SBP, *z* score	−0.54 (−1.01–0.31)	−0.38 (−0.72–0.04)	−0.68 (−0.80–0.34)	0.826
DBP, *z* score	0.63 (0.14–1.07)	0.73 (−0.15–1.05)	0.98 (0.73–1.62)	0.315
Duration of diabetes, years		4.90 (1.25–10.18)	3.00 (1.00–9.30)	0.494
HbA1C, %		9.21±2.57	10.32±3.03	0.316
Urinary albumin, mg/g creatinine		7.46 (4.72–10.24)	83.80 (44.73–101.45)	<0.001
Serum creatinine (mg/dl)	0.60±0.16	0.73±0.12	0.75±0.18	0.001*, 0.929**
eGFR, ml/min/1.73 m^2	106.66±20.73	93.41±13.29	89.15±28.17	0.006*, 0.579**
CRP, mg/dl	0.68±0.90	0.66±0.88	0.55±0.56	0.948
Total cholesterol, mg/dl		168.03±35.63	201.76±30.06	0.02
LDL, mg/dl		97.11±36.06	117.22±21.85	0.151
HDL, mg/dl		53.33±11.65	66.13±21.34	0.06

Data are expressed as the mean±SD for parametric variables and as the median (interquartile range) for nonparametric variables.*BMI* body mass index, *CRP* C-reactive protein, *Cr* creatinine, *DBP* diastolic blood pressure, *eGFR* estimated glomerular filtration rate, *Hb* hemoglobin, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *MA* microalbuminuria, *NA* normoalbuminuria, *SBP* systolic blood pressure

* *p* value, control group vs. patient group (NA and MA groups combined)

** *p* value, NA group vs. MA group

Correlations between urinary L-FABP and serum/urinary YKL-40 levels and other clinical and laboratory parameters

There were no significant correlations between urinary L-FABP or serum/urinary YKL-40 levels and urinary ACR (Table 4). Nor were there any correlations found between the L-FABP or YKL-40 levels, and other clinical or laboratory parameters including eGFR, systolic and diastolic blood pressures, age (at the time of being diagnosed with diabetes initially and at the time of taking a urine sample) and the duration of diabetes (data not shown). However, the urinary L-FABP/Cr level was significantly correlated with the HbA1C level (p=0.005, r=0.511) and the urinary YKL-40/Cr ratio (p=0.043, r=0.260). As shown in Table 5, the urinary L-FABP/Cr ratio was significantly higher in patients with>8.0 % HbA1C than in those with≤8.0 % HbA1C (p=0.009).

Discussion

This study is the first to demonstrate the associations of urinary L-FABP and YKL-40 among patients with childhood-onset T1D. The concentrations of urinary YKL-40/Cr were

Table 2 Serum and urinary levels of YKL-40 and urinary levels of L-FABP in children with T1D and in healthy controls

	Control (n=32)	Total patients (n=29)	NA group (n=20)	MA group (n=9)
sYKL-40 (ng/ml)	20.5 (14.48–32.66)	21.67 (18.50–27.83)	21.41 (18.43–26.65)	23.89 (19.30–33.41)
uYKL-40/Cr (ng/mg)	0.005 (0.002–0.27)	0.55 (0.36–0.99)*	0.67 (0.47–1.03)**	0.52 (0.18–0.79)***
uL-FABP/Cr (ng/mg)	2.91 (1.72–4.41)	4.32 (1.56–9.82)	6.3 (2.10–36.60)	9.82 (4.32–13.89)***,****

Data are expressed as the median (interquartile range) for nonparametric variables

sYKL-40 serum YKL-40, *uYKL-40/Cr* urinary YKL-40/creatinine ratio; *uL-FABP/Cr* urinary liver-type fatty acid binding protein/creatinine ratio; *NA* normoalbuminuria; *MA* microalbuminuria

**p*<0.05, control vs. total patient group, ***p*<0.05, control vs. NA group, ****p*<0.05, control vs. MA group, *****p*<0.05, NA group vs. MA group

Table 3 Comparisons of scores for urinary markers between children with T1D and healthy controls

	Control (n=32)	Total patients (n=29)	Normoalbuminuria group (n=20)	Microalbuminuria group (n=9)
Score for uYKL-40/Cr, score (n)	0 (24), 1 (8)	0 (6), 1 (15), 2 (8)*	0 (3), 1 (11), 2 (6)**	0 (3), 1 (4), 2 (2)***
Score for uL-FABP/Cr, score (n)	0 (24), 1 (6), 2 (2)	0 (15), 1 (6), 2 (8)*	0 (12), 1 (5), 2 (3)	0 (3), 1 (1), 2 (5)***
Sum of scores, score (n)	0 (18), 1 (10), 2 (4)	0 (3), 1 (9), 2 (10), 3 (6). 4 (1)*	0 (2), 1 (7), 2 (7), 3 (4)**	0 (1), 1 (2), 2 (3), 3 (2), 4 (1)***

Data are expressed as frequencies for categorical variable

Score of 0, uYKL-40/Cr or uL-FABP/Cr levels<the upper quartile value of the control group; Score of 1, the upper quartile value of the control group≤ uYKL-40/Cr or uL-FABP/Cr levels<the upper quartile value of diabetic patient groups (normo and microalbuminuria groups); Score of 2, uYKL-40/Cr or uL-FABP/Cr levels≥the upper quartile value of patient groups; Sum of scores, Score of uYKL-40/Cr+Score of uL-FABP/Cr

Key: *uYKL-40/Cr* urinary YKL-40/creatinine ratio, *uL-FABP/Cr* urinary liver-type fatty acid binding protein/creatinine ratio

*p<0.05, control vs. total patients group, **p<0.05, control vs. normoalbuminuria group, ***p<0.05, control vs. microalbuminuria group

elevated in the patients with diabetes, even in the NA group compared with control subjects. On the other hand, the concentrations of urinary L-FABP/Cr were elevated in the MA group compared with the NA group.

Previous studies have shown the associations of YKL-40 or L-FABP with diabetic nephropathy. L-FABP is a marker for tubular injury, and several experimental studies and clinical studies on adult patients with diabetes have consistently supported this [8, 27, 28]. Our results also indicate that urinary L-FABP is a tubular injury marker for early DN in pediatric patients with T1D by showing that urinary L-FABP levels were increased in the MA group compared with the control subjects and the NA group, and some patients with normoalbuminuria had higher levels of urinary L-FABP/Cr than those in patients with microalbuminuria. However, the degree of albuminuria (ACR) and the urinary levels of L-FABP/Cr were not significantly associated and the levels of L-FABP/Cr did not differ statistically between the patients with normoalbuminuria and the control group. The reason for this is unclear. However, a recent large-scale study of patients with type 2 diabetes by Kamijo et al. [28] showed similar findings, i.e., although the urinary L-FABP level was significantly correlated with the urinary albumin level in all of the patients (n=104), there was no correlation in the subgroup of patients who had an eGFR >60 ml/min/1.73 m^2 (n=59). In addition, Vaidya et al. suggested that microalbuminuria in the absence of significant tubular injury might be a more reversible state by demonstrating that low baseline concentrations of tubular injury markers such as urinary kidney injury molecule (KIM-1) and *N*-acetyl-β-D-glucosaminidase (NAG) in type 1 diabetic patients were significantly associated with the regression of microalbuminuria over the subsequent 2 years [29]. Our study was a cross-sectional study with relative small number of subjects and further studies including large numbers of subjects with various degrees of albuminuria and longitudinal observations are needed to confirm the association of urinary L-FABP with renal pathophysiological condition in pediatric patients with T1D.

In our study, urinary L-FABP/Cr levels showed significant correlations with HbA1C levels (p=0.005), although the ACR did not. In addition, as shown in Table 5, urinary L-FABP/Cr levels in the group with poor glycemic control (HbA1C≥8 %) were higher than in the group with good glycemic control (HbA1C<8 %). Previous studies have suggested that hyperglycemia affects the proximal tubule directly, and that the degree of tubular proteinuria is related to glycemic control [30, 31]. Moreover, the application of an SGLT2 inhibitor not only reduced the HbA1C levels but also attenuated renal hyperfiltration in patients with diabetes, likely by affecting tubuloglomerular feedback mechanisms, showing renoprotective effects [32, 33]. These associations with the degree of glycemic control and tubular injury remain to be determined clearly but might help to identify modifiable risk factors for the development and progression of DN and

Table 4 The associations between serum or urinary YKL and urinary L-FABP levels, and the albumin-to-creatinine ratio (ACR) and HbA1C levels in pediatric patients with T1D (n=29)

	ACR Spearman's r (p)	HbA1C
ACR		0.199 (0.300)
Serum YKL-40 (ng/ml)	0.183 (0.341)	0.133 (0.492)
Urinary YKL-40/Cr (ng/mg)	−0.064 (0.741)	−0.003 (0.988)
Urinary L-FABP/Cr (ng/mg)	0.223 (0.245)	0.511 (0.005)
Urinary YKL-40/Cr and urinary L-FABP/Cr	0.260 (0.043)	

L-FABP liver type fatty acid binding protein, *T1D* type 1 diabetes

Table 5 The median (interquartile range) concentrations of YKL-40 and L-FABP according to HbA1C levels in pediatric patients with T1D

	HbA1C<8.0 % (n=11)	HbA1C≥8.0 % (n=18)	p value
Serum YKL-40 (ng/ml)	21.15 (18.23–27.02)	23.16 (19.69–32.01)	0.387
Urinary YKL-40/Cr (ng/mg)	0.55 (0.45–0.78)	0.67 (0.22–2.04)	0.674
Urinary L-FABP/Cr (ng/mg)	1.64 (1.21–3.32)	5.83 (3.38–13.16)	0.009
ACR (mg/g)	7.89 (5.80–13.54)	15.46 (6.33–43.33)	0.438
eGFR (ml/min/1.73 m^2)	90.63 (82.77–97.75)	90.32 (83.39–101.11)	0.948

ACR albumin-to-creatinine ratio, *eGFR* estimated glomerular filtration rate, *L-FABP* liver type fatty acid binding protein, *T1D* type 1 diabetes

subsequently find novel therapeutic strategies such as SGLT2 inhibitors for preventing or delaying the progression of DN.

The cut-off value of urinary L-FABP for defining renal injury or predicting the progression of nephropathy in children has not been determined. Almost all published reports on urinary L-FABP in children have been performed on patients of young age with congenital heart disease receiving cardiopulmonary bypass surgery [34, 35]. They already had ischemic injury, so the baseline levels of urinary L-FABP of these patients were high. Therefore, they cannot be used for reference values of urinary L-FABP in children without congenital heart disease. Based on the results for the cut-off value of urinary L-FABP for adult control subjects (n=412; 8.4 ng/mg of urinary L-FABP/Cr) [28], there were three patients (3/20, 15 %) in the NA group and five patients (5/9, 56 %) in the MA group who had urinary L-FABP/Cr levels >8.4 ng/mg in our study population. Considering that L-FABP is an independent marker for reflecting tubulointerstitial injury and predicting the progression of DN, and tubular injury might be the main factor involved in the development and progression of DN [36, 37], close monitoring of renal function for these patients might be needed.

YKL-40 is a marker for chronic inflammation and endothelial dysfunction [20]. Several studies have consistently shown that plasma YKL-40 levels are elevated in adult patients with type 1 or 2 diabetes compared with those of control subjects, and the levels were positively correlated with the degree of albuminuria [20, 21, 38]. In our study, plasma YKL-40 levels were not different between groups, but urinary YKL-40 levels were significantly elevated in patient groups compared with the control group. To the best of our knowledge, there has been only one study regarding the association of urinary YKL-40 and diabetes. JH Lee et al. showed that the concentrations of urinary YKL-40 were elevated in type 2 diabetes patients with macroalbuminuria compared with those of control subjects, but not elevated in patients with normo or microalbuminuria [26]. As a mechanism for their results, they suggested that reduced tubular reabsorption of filtered YKL-40 due to tubular injury or increased renal production of YKL-40 due to localized inflammation may lead to increased urinary excretion of YKL-40 in macroalbuminuric diabetic patients. We speculate similar mechanisms in the pathogenesis of renal injury in childhood-onset T1D regarding the role of urinary YKL-40. However, the results for urinary YKL-40/Cr were not consistent with those for urinary L-FABP/Cr, a marker for tubular injury. Moreover, as shown in Table 3, the combined scores for urinary YKL-40/Cr and urinary L-FABP/Cr did not have the synergistic effect compared with the each score for urinary YKL-40/Cr or L-FABP/Cr. Taken together, these two markers may have different roles in the development of renal injury in childhood-onset T1D. Therefore, we may suggest that increased urinary YKL-40 may be the result of localized inflammation rather than tubular damage. Previous studies have demonstrated the role of persistent localized inflammation for renal injury [39, 40]. Yung et al. reported that despite clinical remission, ongoing inflammatory processes may persist within the renal parenchyma leading to further kidney injury [41]. Our study is a cross-sectional study, therefore we do not know whether the persistence of localized inflammation representing persisting elevated urinary YKL-40 levels may be associated with tubular injury representing elevated urinary L-FABP levels in childhood-onset T1D, though the urinary YKL-40/Cr levels were correlated with the urinary L-FABP/Cr levels (p=0.043, r=0.260). Further evaluations are needed to clarify the role of urinary YKL-40 for renal injury in DN.

The cut-off value of urinary YKL-40 for defining renal injury has not been clearly established. Based on the results of few studies evaluating urinary YKL-40 as a marker for tubular injury, the levels of urinary YKL-40/Cr in control subjects were very low and on the other hand, the levels in patients having renal injury were markedly elevated [23, 26]. Our results were consistent with these previous studies. However, to determine whether urinary YKL-40 is a reliable marker for renal injury, further studies are needed, especially in a population of children.

Our study has some limitations to consider. First, it was conducted with a cross-sectional design and the number of subjects was relatively small. Therefore, longitudinal observations in a larger population will be needed to determine whether patients with increased urinary L-FABP or YKL-40 levels are at risk for a decline in renal function or at risk for the progression of albuminuria independent of their albuminuria status. Second, to strengthen the role of urinary

L-FABP and urinary YKL-40 as markers for tubular injury in DN, it will be necessary to evaluate the correlations with other tubular injury markers such as urinary neutrophil-gelatinase-associated lipocalin, β_2 microglobulin or *N*-acetyl-beta-D-glucosaminidase or other inflammatory markers such as interleukin-6 or tumor necrosis factor-α. Third, our diabetic patients did not show hyperfiltration, although they had preserved renal function. The reason was not clear. However, as an estimate for the reasons, the formula for estimated GFR in the present study was the creatinine-based, which may be less accurate than that of cystatin-C based [42, 43]. In addition, the creatinine levels of diabetic patients in our study were similar to those in other studies on adolescent diabetic patients [44]. Fourth, some baseline characteristics between each group were different, which may affect the concentrations of urinary YKL-40/Cr or L-FABP/Cr. Subjects in the patient groups were older than subjects in the control group. However, no association was found between the age and urinary YKL-40/Cr or L-FABP/Cr levels, and the total cholesterol levels were significantly higher in the microalbuminuria group than in the normoalbuminuria group and the ranges for the duration of diabetes were wide (median (IQR)-4.2 year (1–9.8 year)). However, other metabolic risk factors including blood pressures, BMI and LDL levels were not different between NA and MA groups. In addition, the levels of urinary YKL-40/Cr or L-FABP/Cr did not differ according to their cholesterol level (≥200 mg/dl or<200 mg/dl) or the duration of diabetes (≥5 years or<5 years) (data not shown). Therefore, we assume that the cholesterol level or the duration of diabetes were not significantly affecting to the levels of urinary YKL-40 or L-FABP.

Currently, albuminuria reflecting glomerular injury has been used as an early clinical marker for diagnosing DN. However, the degree of albuminuria did not always correlate with the pathologic change or renal outcome [2, 45]. Moreover, current therapeutic strategies including applying inhibitors of the renin–angiotensin–aldosterone system when microalbuminuria persists for several months have shown disappointing results for preventing the progression of DN [46, 47]. As a result, there is a continuing need for early markers of renal injury. As part of its efforts, the present study suggests that urinary L-FABP/Cr and YKL-40/Cr may reflect renal injury in early stages of nephropathy in patients with childhood-onset type 1 diabetes, even in the normoalbuminuric state.

In conclusion, this is the first report of the association between urinary YKL-40/Cr representing localized renal inflammation and L-FABP/Cr representing tubular injury, and childhood-onset T1D. In this patient population, further studies are needed to investigate the pathophysiological role of these biomarkers in the development and progression of renal injury in order to better elucidate their potential value as therapeutic targets.

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Ethics statement This study was approved by the institutional review board of the Catholic University of Korea and written informed consent was obtained from all study participants and the caregivers of each child before enrollment.

Conflict of interest The author(s) declare that they have no competing interests.

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