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Spot Urine Albumin to Creatinine Ratio and Serum Cystatin C are Effective for Detection of Diabetic Nephropathy in Childhood Diabetic Patients

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Spot urinary albumin to creatinine ratio (ACR) measurement has been suggested as a surrogate to 24-hr urine collection for the assessment of microalbuminuria, and cystatin C (cysC) is known as an advantageous marker for renal function. The aim of this study was to evaluate the clinical values of spot urinary ACR and serum cysC for the assessment of diabetic nephropathy instead of 24-hr urine microalbumin in children and adolescents with diabetes. A total of 113 children and adolescents (age 12-19 yr, M:F = 47:66) with type 1 or 2 diabetes were enrolled. We evaluated the validity of spot urine ACR and serum cysC, and then compared them to 24-hr urine microalbumin and creatinine clearance. Spot urine ACR was correlated with 24-hr urine albumin excretion (R^2 = 0.828, P = 0.001) and creatinine clearance (R^2 = 0.249, P = 0.017). The ROC curve analysis of serum cysC demonstrated higher diagnostic accuracy than that of serum creatinine (AUC 0.732 vs 0.615). Both the measurements of spot urine ACR and serum cysC might better predict the presence of diabetic nephropathy than 24-hr urine microalbumin in childhood diabetic patients.

Key Words: Diabetic Nephropathies; Albumin to Creatinine Ratio; Cystatin C; Childhood Diabetes

INTRODUCTION

Diabetic nephropathy is a significant complication in diabetic patients, and it is becoming the most common cause of end stage renal disease (1). Childhood diabetic patients live long enough for nephropathy to develop because they develop diabetes early, usually before reaching adolescence. Therefore, preventing diabetic nephropathy or delaying the disease progression by way of early detection is very important (2, 3).

The determination of microalbuminuria has been suggested as an early predictor of diabetic nephropathy. The gold standard for measuring urine albumin excretion is still 24-hr urine collection. However, the standard clearance technique necessitates timed urine collection, which is not only time-consuming but also subject to error. Particularly, children with diabetes tend to collect 24-hr urine inaccurately because they are young and immature. Furthermore, 24-hr urinary albumin excretion rates can be altered by various conditions, such as intra-individual variability and day-to-day variation during the evolution of albuminuria (4).

Recently, the albumin to creatinine ratio (ACR), measured from a random urine sample, was suggested to be an effective surrogate to 24-hr urine collection for detecting microalbumin-

uria (5-7). ACR is convenient to perform, and is less affected by variation in urine concentration because it is a ratio between two measured substances.

Also, serum creatinine has been widely used as a marker of GFR, but it is not sensitive enough to detect decreased renal function. Therefore, various plasma low molecular weight proteins have been suggested as valuable markers of decreased renal function in place of serum creatinine (8). Among these markers, previous studies demonstrated that serum cystatin C (cysC) might be a superior marker for the evaluation of renal function than serum creatinine (9). However, the effectiveness of cysC for estimating GFR has not been sufficiently demonstrated in children with diabetes.

Therefore, the aim of this study was to investigate whether spot urine ACR and serum cysC are accurate and effective in assessing renal function instead of 24-hr urine microalbumin in children and adolescents with diabetes.

MATERIALS AND METHODS

A total of 113 children and adolescents (age 12-19 yr) with type 1 or 2 diabetes at Childhood Diabetes Clinic of Severance Children's Hospital were included in this study from January 2008

to August 2010. Ninety-eight (41 males and 57 females) patients were type 1 and 15 (6 males and 9 females) were type 2. Age, duration of disease, mean glycated hemoglobin (HbA1c), 24-hr urine microalbumin, ACR in spot urine, serum Cr, serum cysC, high sensitivity C-reactive protein (CRP), which rises in response to inflammation, were checked in all patients. Creatinine clearance was calculated using 24-hr urine collection. Estimated GFR (eGFR) was calculated with the Schwartz formula for patients under the age of 18 yr: eGFR (mL/min/1.73 m^2) = k $*$ Height (cm)/serum Cr (mg/dL) (k = 0.55 in children to 13 yr of age, k = 0.70 in adolescent males [the constant remains 0.55 for females]) (10) and Modification of Diet in Renal Disease (MDRD) formula for those of ages of 18 to 19 : eGFR = 186 $*$ (Serum Cr) - 1.154 $*$ (Age) - 0.203 $*$ (0.742 if female) (11).

Serum creatinine was determined by an enzymatic method (Kodak Ektachem 700 XR-C system, Eastman Kodak, Rochester, NY, USA). Serum cysC was measured by automated particle-enhanced immunonephelometry using a BN100 nephelometer (Dade Behring, Marburg, Germany).

Data were analyzed by the SAS program (version 9.1; SAS Institute, Cary, NC, USA). We used the generalized linear regression model and chi-squared test to verify the demographic trends of continuous and categorical variables over the ordinal variable of time, respectively. Significance was determined as $P < 0.05$. The sensitivity and specificity of serum cysC, serum creatinine, and creatinine clearance for the detection of reduced GFR were assessed by the ROC curve.

Ethics statement

This study was approved by the Institutional Review Board of Yonsei University Severance Hospital (IRB number 4-2012-0001). Written informed consent was exempted from all subjects as well as their parents.

RESULTS

Baseline patient characteristics are shown in Table 1. There were no significant differences between the type 1 and 2 diabetes groups regarding mean HbA1c, serum creatinine, cysC and creatinine clearance. Ninety six patients (85%) had normoalbuminuria, 17 (15%) had microalbuminuria and no patient had macroalbuminuria. We compared the microalbuminuria group with the normoalbuminuria group (Table 2). Age, duration of disease, and mean HbA1c levels were significantly higher in the microalbuminuria group than in the normoalbuminuria group, suggesting that the incidence of diabetic nephropathy increases according to the progress of the disease and poor glycemic control. Serum creatinine level was not significantly different between the two groups, but serum cysC was significantly higher in the microalbuminuria group ($P = 0.040$). Creatinine clearance was significantly lower in the microalbuminuria group. High sensitivity CRP did not differ between the two groups. Spot urine ACR was positively correlated with 24-hr urine albumin excretion ($R^2 = 0.828$ and $P = 0.001$) (Fig. 1) and negatively correlated with creatinine clearance ($R^2 = 0.249$ and $P = 0.017$).

We analyzed serum creatinine, cysC and spot urine ACR between the patients with creatine clearance < 60 mL/min/1.73 m^2 (group I) and those with creatine clearance > 60 mL/min/1.73 m^2 (group II) (Table 3). Serum creatinine did not differ between the two groups, but spot urine ACR and serum cysC were significantly greater in group I.

Table 2. Comparison between the normoalbuminuria and microalbuminuria groups

Parameters	NormoA (n = 96)	MicroA (n = 17)	P value
Type 1/2 diabetes	83/13	15/2	
Male/female	40/56	7/10	
Age (yr)	15.7 ± 4.7	17.2 ± 3.2	0.026
Duration (yr)	6.5 ± 3.8	9.1 ± 5.2	0.012
HbA1c (%)	7.6 ± 2.8	8.9 ± 3.6	0.037
Serum creatinine (mg/dL)	0.76 ± 0.21	0.92 ± 0.34	0.066
Serum cystatin C (mg/L)	0.52 ± 0.29	0.69 ± 0.33	0.040
Creatinine clearance (mL/min/1.73 m^2)	83.7 ± 31.7	62.9 ± 36.3	0.017
Spot urine ACR (mg/g)	14.4 ± 12.9	77.8 ± 34.3	0.001
24-hr urine microalbumin (mg/day)	7.8 ± 9.2	65.9 ± 34.9	0.001
hs CRP (mg/dL)	0.57 ± 0.48	0.62 ± 0.37	0.227

NormoA, Normoalbuminuria; MicroA, Microalbuminuria; HbA1c, Glycated hemoglobin; ACR, Albumin to creatinine ratio; hs CRP, high sensitivity C-reactive protein.

Table 1. Baseline characteristics of patients with type 1 or 2 diabetes

Parameters	Type 1 DM (n = 98)	Type 2 DM (n = 15)	Total (n = 113)	P value
Male/female	41/57	6/9	47/66	
Age (yr)	15.7 ± 4.0	16.3 ± 3.2	15.9 ± 3.7	0.435
Duration (yr)	7.1 ± 5.1	3.8 ± 3.1	6.7 ± 4.7	0.012
HbA1c (%)	8.4 ± 2.7	7.5 ± 2.8	8.1 ± 2.7	0.129
Serum creatinine (mg/dL)	0.81 ± 0.34	0.89 ± 0.42	0.83 ± 0.37	0.384
Serum cystatin C (mg/L)	0.55 ± 0.28	0.60 ± 0.35	0.57 ± 0.30	0.205
Creatinine clearance (mL/min/1.73 m^2)	80.3 ± 32.9	74.1 ± 34.2	79.1 ± 35.6	0.098
Spot urine ACR (mg/g)	24.8 ± 20.8	26.3 ± 18.3	25.2 ± 20.1	0.132
24-hr urine microalbumin (mg/day)	20.4 ± 19.4	22.1 ± 16.7	23.1 ± 18.6	0.167
hs CRP (mg/dL)	0.49 ± 0.34	0.58 ± 0.50	0.53 ± 0.44	0.089

HbA1c, Glycated hemoglobin; ACR, Albumin to creatinine ratio; hs CRP, High sensitivity C-reactive protein.

Table 3. Comparison of serum parameters between the creatinine clearance < 60 and creatinine clearance > 60 groups

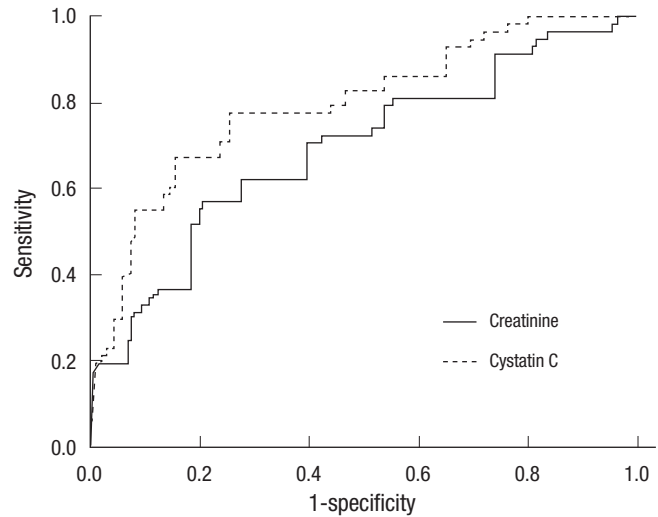| Parameters	CrCl < 60 (mL/min/1.73 m²)	CrCl > 60 (mL/min/1.73 m²)	P value
No.	20	93	
Serum creatinine (mg/dL)	0.97 ± 0.49	0.76 ± 0.31	0.058
Serum cystatin C (mg/L)	0.68 ± 0.35	0.49 ± 0.27	0.024
Spot urine ACR (mg/g)	42.8 ± 34.9	21.8 ± 20.4	0.019

CrCl, Creatinine clearance; ACR, Albumin to creatinine ratio.
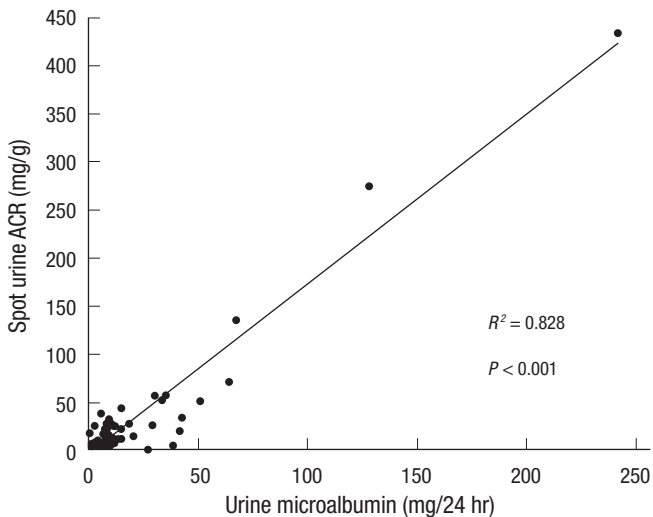
Fig. 1. Correlation between 24-hr microalbumin (mg/24 hr) and spot urine albumin to creatinine ratio (ACR) (mg/g). The lower cutoff for microalbuminuria; 30 (mg/24 hr) in 24-hr urine microalbumin represents 36 (mg/g) in spot urine ACR.

Fig. 2. Serum cystatin C showed better diagnostic accuracy than serum creatinine at the cutoff value of below 60 mL/min/1.73 m² of creatinine clearance.

The sensitivity and specificity of serum cysC and creatinine compared with creatinine clearance were estimated via ROC curves (Fig. 2). The area under the curves (AUC) with the cut-off value of 60 mL/min/1.73 m² was 0.732 for serum cysC and 0.615 for serum creatinine. The AUC was significantly higher for serum cysC ($P = 0.028$), but not for serum creatinine ($P = 0.069$). The sensitivity and specificity of cysC were 87.3% and 66.2%, respectively, with the upper reference limit as the cut-off. However, serum creatinine showed 74.3% and 58.3%, respectively. The ROC curve analysis of serum cysC demonstrated higher diagnostic accuracy than that of serum creatinine.

DISCUSSION

The gold standard for the assessment of diabetic nephropathy in children is still the quantitative analysis of microalbumin through 24-hr urine collection. However, many factors such as exercise, hypertension, hyperglycemia can influence daily urinary albumin excretion (12). Day-to-day variation according to the volume of collected urine can hinder the diagnosis of diabetic nephropathy (4). Discovery of microalbuminuria through 24-hr urine collection can also be erroneous because of improper collection, which commonly happens with children and

adolescents. Therefore, a test using timed urine collection has been suggested to be repeated three times to confirm microalbuminuria, but this is very hard to apply to children.

Recent studies have demonstrated good correlation between spot urine ACR and 24-hr urinary albumin excretion (6, 13). Spot urine ACR was thought to be more accurate because it eliminates the possibility of improper collection. The method using spot urine samples may ensure better compliance than timed urine test, especially in childhood patients. Furthermore, the spot urine ACR has the advantage of being less affected by urine volume because it is a ratio of two measured substances (14).

Nevertheless, few studies have reported the efficacy and effectiveness of spot urine ACR compared with creatinine clearance through 24-hr urine collection in childhood patients with diabetes. The present study showed that spot urine ACR was closely correlated with 24-hr urine albumin excretion and creatinine clearance in children and adolescents with diabetes. However, we can not definitively support that spot urine ACR should be used in place of 24-hr urine collected miacroalbumin due to the small study population. We do, however, feel that spot urine ACR would be useful as a screening tool when timed urine collection is diffcult to obtain in out-patient settings.

The use of endogenous markers to evaluate renal function is also important in the evaluation of diabetic nephropathy. Various low molecular proteins, such as cysC, β_2-microglobulin, and collagen type IV, instead of serum creatinine have been suggested as useful endogenous markers for evaluating renal function (8). Among these, previous studies have demonstrated that cysC might be a more sensitive indicator of GFR than serum creatinine, but there have been few studies on cysC as an endogenous marker reflecting GFR in children with diabetic nephropathy (15-17).

In the present study, the level of serum cysC, but not serum

creatinine, was significantly different between the normoalbuminuria group and the microalbuminuria group. Estimated GFR according to the Schwartz and MDRD II formulas did not demonstrate any difference between the two groups, although creatinine clearance was significantly lower in the microalbuminuria group. It is thought that serum creatinine is unable to reflect the early signs of decreased renal function in childhood diabetic patients, while serum cysC can. However, we cannot say that serum cysC might be surrogate marker of diabetic nephropathy, because diabetic patients have the increased possibility of decreased renal function due to causes other than diabetic nephropathy. Nevertheless, cysC based GFR is suggested to be a useful method for estimating renal function (18). Recently, Schwartz et al. proposed an updated Schwartz formula using cysC (19). We can estimate that cysC based GFR may be a better method than creatinine based GFR on the findings in this study.

Our studies have some limitations in that we did not perform the measurement of exogenous substances such as insulin, ^{51}Cr-EDTA, ^{99m}Tc-diethylenetriaminepentaacetic acid, iohexol (20) or DTPA renogram, which has been suggested as the most accurate method to evaluate renal function. However, DTPA renogram or other accurate measurements based on the measurement of exogenous substances are hard to perform, especially in childhood diabetic patients. More studies with a large sample population are needed to confirm that spot urine ACR can replace 24-hr urine microalbumin in childhood diabetes.

There was a discrepancy between eGFR and creatinine clearance through 24-hr urine collection in this study. For this we considered two possibilities. First, the discrepancy may implicate that urine collection is inaccurate. Second, the MDRD formula within the normal range of creatinine might not reflect accurate renal function.

Nevertheless, to the best of our knowledge, the present study is the first to simultaneously check both serum cysC and spot urine ACR in children and adolescents with diabetes, and our study demonstrated that spot urine ACR might be a more accurate, convenient, and effective indicator for the detection of microalbuminuria instead of 24-hr urine microalbumin in diabetic children. CysC based GFR may be a more accurate method than creatinine based GFR to evaluate renal function.

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