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Serum Cystatin C Assay for the Detection of Early Renal Impairment in Diabetic Patients

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The ability to assess renal function in diabetes patients rapidly and early is of major importance. This study was designed to determine whether cystatin C can replace serum creatinine as the screening marker for reduced glomerular filtration rate (GFR) in type 2 diabetes patients. The study was performed on 51 type 2 diabetic patients. GFR was estimated by the plasma clearance of ^{99m}Tc-DTPA. The correlation between ^{99m}Tc-DTPA clearance and levels of serum cystatin C, serum creatinine, and creatinine clearance was determined. Sensitivity and specificity for the diagnosis of renal impairment (defined as GFR $<$ 68 ml/min) were calculated by a receiver operating characteristic (ROC) curve for serum cystatin C, serum creatinine, and creatinine clearance. The correlation coefficients with ^{99m}Tc-DTPA clearance were -0.744 for serum cystatin C, -0.658 for serum creatinine, and +0.625 for creatinine clearance ($P < 0.001$). With a cutoff value of 68 mL/min, the area under the ROC curve (AUC) was 0.891 for cystatin C, 0.77 for creatinine, and 0.753 for creatinine clearance. The AUC was statistically different between serum cystatin C and creatinine clearance ($P < 0.05$). The ROC plot indicates that cystatin C is superior to serum creatinine and creatinine clearance for detecting impaired GFR. Serum cystatin C appropriately reflects GFR in diabetes, and is more efficacious than serum creatinine and creatinine clearance in detecting reduced GFR in type 2 diabetes patients. J. Clin. Lab. Anal. 18:31–35, 2004.

Key words: cystatin C; type 2 diabetes; serum creatinine; glomerular filtration rate; creatinine clearance; receiver operating characteristic

INTRODUCTION

Diabetic nephropathy is one of the most serious complications in type 2 diabetes mellitus, and is frequently seen in patients with end-stage renal diseases. Modern treatments have greatly improved the life span of such patients. On the other hand, the increase of chronic complications (such as diabetic nephropathy and renal failure) occurring in diabetic patients presents a challenge to clinical practitioners. In clinical practice, progressive kidney failure often goes unrecognized until a patient has lost $>50\%$ of normal kidney function. This is in part because of the lack of an easy method to measure the glomerular filtration rate (GFR) in a clinical setting. Many techniques have been used to detect early renal impairment in diabetes and other diseases. Microalbuminuria is commonly seen in patients with type 2 diabetes. The albumin excretion rate (AER) is a useful indicator, but a day-to-day variation of up to 40% limits its use for that purpose (1). GFR using exogenous substances, such as inulin, ^{51}Cr-EDTA, and ^{125}I-iothalamate, is considered to be the gold standard, but is rarely used clinically because of the cost and the cumbersome procedures involved. Creatinine clearance used for the measurement of GFR requires 24-hr urine samples; it is also complicated and has a high error rate. Serum creatinine is widely used for the rapid assessment of GFR; however, GFR may be inadequately estimated due to differences in sex and muscle mass, tubular secretion of creatinine, and the interference of serum noncreatinine substances (such as VitC, glucose, acetone) to Jaff's kinetic method (2). Human cystatin C is a nonglycosylated, low-molecular-weight, basic protein that belongs to the superfamily of cysteine proteinase inhibitors. It is steadily expressed in most tissues, and is present at relatively high concentrations in body fluids. In the kidney, it is freely filtered through glomeruli, and is completely reabsorbed and

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catabolized in proximal tubules (3). The characteristics of cystatin C have made it an endogenous marker for GFR assessment. Many studies have reported that it is more sensitive than creatinine for the evaluation of early renal impairment (4,5); however, several studies showed opposite results (6). We used plasma ^{99m}Tc-DTPA clearance as the reference of relatively accurate GFR to evaluate serum cystatin C, serum creatinine, and creatinine clearance in the detection of reduced GFR in 51 diabetic patients.

MATERIALS AND METHODS

Patients and Samples

Blood samples were obtained from 51 diabetic patients (25 males and 26 females, 53.7 ± 10.34 years old) who had been admitted to the Department of Endocrinology, Peking University First Hospital. Type 2 diabetes was diagnosed according to the criteria published by WHO in 1999 (7). Patients with malignancies or who were receiving corticosteroid treatment (which can cause changes in serum cystatin C (8)) were excluded from this study. Written informed consent was obtained from the patients before the test. Blood samples were taken <1 day before or after the ^{99m}Tc-DTPA clearance test, and the sera were stored at -20°C until they were used.

Measurement of ^{99m}Tc-DTPA Clearance, Serum Cystatin C, Serum Creatinine, and Creatinine Clearance

GFR was measured by the clearance of plasma ^{99m}Tc-DTPA. After the patient was given a single bolus intravenous injection of 185MBq ^{99m}Tc-DTPA, the ^{99m}Tc-DTPA radioactivity was recorded by a probe set on the patient's lower back, and was measured in a GE Starcame 300 gamma counter with an energy window of 140 keV. Serum cystatin C was measured by particle-enhanced immunonephelometry using a BN100 nephelometer (Dade Behring, Marburg, Germany). Serum and urine creatinine were measured by Jaff's kinetic method on an Hitachi 7170 (Hitachi Company, Japan) autoanalyzer. Creatinine clearance was calculated from serum creatinine and creatinine in 24-hr urine. Normal ranges in this hospital are 44–133 μmol/L for serum creatinine and >68 mL/min for ^{99m}Tc-DTPA clearance.

Statistical Analysis

Statistics were analyzed with SPSS 10.0 (SPSS, Chicago, IL) and MedCalc version 6 (Medcalc, Mariakerke, Belgium). Correlations between ^{99m}Tc-DTPA clearance and the three parameters were calculated by the Pearson coefficient. $P<0.05$ was considered to be significant. The sensitivity and specificity of serum cystatin C, serum creatinine, and creatinine clearance for the detection of reduced GFR were assessed by the ROC curve. The AUCs were calculated and compared ($P<0.05$ indicated significant difference). Mean concentrations between the two groups were compared using an independent sample t-test. Data are presented as mean $\pm$ SD.

RESULTS

Based on ^{99m}Tc-DTPA clearance, the 51 diabetic patients were divided into two groups: 1) the reduced group (GFR ≤ 68 mL/min, 13 patients) and 2) the normal group (GFR >68 mL/min, 38 patients). Their serum cystatin C, serum creatinine, and creatinine clearance values are summarized in Table 1. Mean serum cystatin C was 1.61 ± 0.57 mg/L in the reduced group and 0.94 ± 0.23 mg/L in the normal group ($P<0.001$). Mean serum creatinine was 106.09 ± 44.69 μmol/L in the reduced group, and 74.37 ± 11.08 μmol/L in the normal group ($P<0.05$). Mean creatinine clearance was 70.19 ± 41.78 mL/min in the reduced group, and 104.18 ± 29.62 mL/min in the normal group ($P<0.05$). In the normal group, serum creatinine was significantly different between males and females (79.89 ± 9.39 μmol/L and 69.11 ± 10.47 μmol/L, respectively; $P=0.002$), but no difference was found in serum cystatin C between males and females (0.92 ± 0.17 mg/L and 0.95 ± 0.27 mg/L, respectively; $P>0.05$). In the reduced group, however, no differences between males and females were found both in serum creatinine (88.57 ± 14.95 μmol/L and 114.24 ± 57.95 μmol/L,

TABLE 1. Mean values of serum cystatin C, serum creatinine, and creatinine clearance in patients with normal and reduced GFR estimated by ^{99m}Tc-DTPA clearance

Group	GFR (ml/min)	No. of patients (male/female)	Age mean (Year)	Serum cystatin C (mg/l)	Serum creatinine (μmol/l)	Creatinine clearance (ml/min)
Reduced	≤ 68	7/6	58.38	1.61 ± 0.57[a]	106.09 ± 44.69[b]	70.19 ± 41.78[b]
Normal	>68	18/20	50.68	0.94 ± 0.23	74.37 ± 11.08	104.18 ± 29.62

[a] $P<0.001$ as compared to the normal group.
[b] $P<0.05$ as compared to the normal group.

respectively; $P > 0.05$) and serum cystatin C (1.34 ± 0.41 mg/L and 1.81 ± 0.58 mg/L, respectively; $P > 0.05$).

The correlation between ^{99m}Tc-DTPA clearance and the three assays in the 51 diabetic patients is shown in Fig. 1. The correlation coefficients were -0.744 ($P < 0.001$) for serum cystatin C, -0.658 ($P < 0.001$) for serum creatinine, and $+0.625$ ($P < 0.001$) for creatinine clearance.

The results of the ROC plot are summarized in Table 2. The AUCs with a cutoff value of 68 mL/min were 0.891 for serum cystatin C, 0.770 for serum creatinine, and 0.753 for creatinine clearance. The AUC was statistically different between serum cystatin C and creatinine clearance ($P < 0.05$), but not between serum cystatin C and serum creatinine ($P > 0.05$) (Fig. 2).

The sensitivity and specificity of the three assays for the diagnosis of reduced GFR were calculated at the determining limits of the ROC curves (Table 2). At the cutoff value of 97.4% for diagnostic specificity, serum cystatin C (61.5%) had higher sensitivity than serum creatinine (38.5%) and creatinine clearance (53.8%). At the cutoff value of 92.3% for diagnostic sensitivity, serum cystatin C (39.5%) also showed higher specificity than creatinine (18.4%) and creatinine clearance (28.9%). These results demonstrate that cystatin C is apparently better than the other two parameters for diagnosing reduced GFR (Table 2).

DISCUSSION

Although cystatin C has been proposed as a reliable serum marker for GFR (9–11), the results in patients with diabetes mellitus have been controversial. A previous study reported that serum cystatin C was a better marker for GFR than creatinine and creatinine clearance in patients with type 2 diabetes (12). However, another study showed that serum cystatin C, as well as serum creatinine and serum β_2-microglobulin, were not sufficiently sensitive to detect early renal failure (6). Our results indicate that the correlation of cystatin C with ^{99m}Tc-DTPA clearance was slightly superior to that of serum creatinine and creatinine clearance. The three parameters were all statistically different between the normal group and the reduced group (Table 1). Although serum cystatin C values were gender-independent, serum creatinine was significantly higher in males

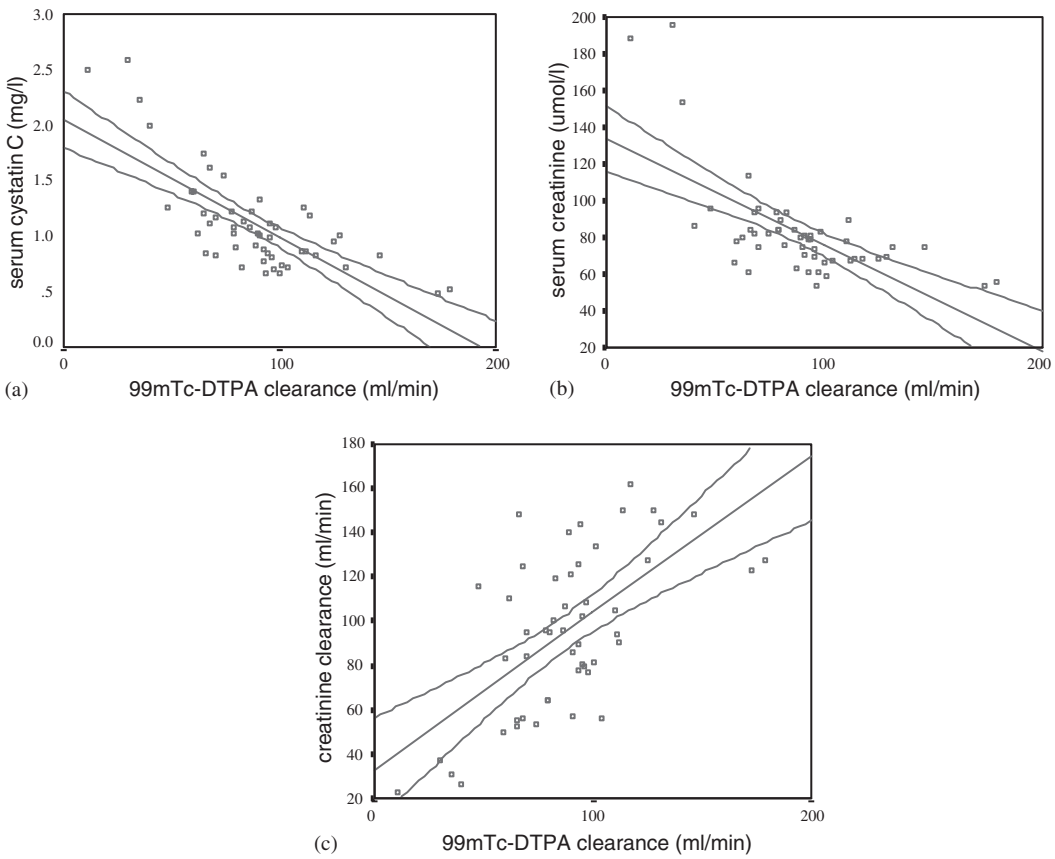

Fig. 1. Correlation between ^{99m}Tc-DTPA clearance and (**a**) serum cystatin C, (**b**) serum creatinine, and (**c**) creatinine clearance assays in 51 type 2 diabetic patients. Correlation coefficients are -0.744 for serum cystatin C, -0.658 for serum creatinine, and $+0.625$ for creatinine clearance.

TABLE 2. Diagnostic accuracy of reduced GFR from serum cystatin C, serum creatinine and creatinine clearance

	Area under the ROC curve, mean ± SD	Sensitivity %	Specificity %
Cys-C (mg/l)	0.891 ± 0.062		
1.02[a]		92.3 (63.9–98.7)	68.4 (51.3–82.5)
0.84[b]		92.3 (63.9–98.7)	39.5 (24.1–56.6)
1.33[c]		61.5 (31.6–86.0)	97.4 (86.1–99.6)
Serum creatinine (μmol/l)	0.770 ± 0.083		
77.6[a]		84.6 (54.5–97.6)	60.5 (43.4–75.9)
63[b]		92.3 (63.9–98.7)	18.4 (7.8–34.3)
94[c]		38.5 (14.0–68.4)	97.4 (86.1–99.6)
Creatinine clearance (min/ml)	0.753 ± 0.071		
80[a]		61.5 (31.6–86.0)	78.9 (62.7–90.4)
125[b]		92.3 (63.9–98.7)	28.9 (15.4–45.9)
55[c]		53.8 (25.2–80.7)	97.4 (86.1–99.6)

[a]Optimum cut-off value (95% confidence intervals [CI] are given in parentheses) result from ROC curve.
[b]Threshold with diagnostic sensitivity of 92.3%.
[c]Threshold with diagnositic specificity of 97.4.

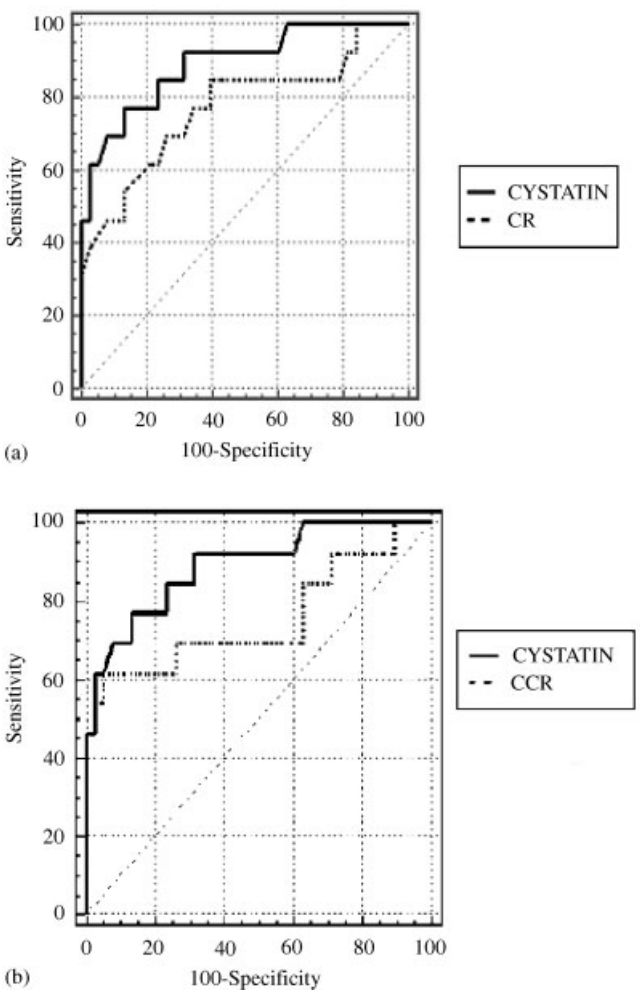

Fig. 2. Nonparametric ROC plots for serum cystatin C (continuous line, AUC = 0.891), (**a**) serum creatinine (CR, dotted line, AUC = 0.770), and (**b**) creatinine clearance (CCR, dotted line, AUC = 0.754).

(11), perhaps because they have greater muscle mass. This means that serum cystatin C is more specific than serum creatinine for evaluating renal function.

In agreement with previous studies in adults (3,9,13), we found that the ROC plot area of serum cystatin C was greater than that of serum creatinine and creatinine clearance (Fig. 2), but the areas for serum cystatin C and serum creatinine were not significantly different ($P > 0.05$). Although cystatin C was approximately equivalent to serum creatinine as a sensitive predictor of reduced GFR, we can see from Table 2 that at the cutoff with a diagnostic specificity of 97.4%, cystatin C (61.5%) had higher sensitivity than serum creatinine (38.5%) and creatinine clearance (53.8%). At the cutoff with a diagnostic sensitivity of 92.3%, cystatin C (39.5%) showed a higher specificity than creatinine (18.4%) and creatinine clearance (28.9%). These results clearly demonstrate that cystatin C is better than other two parameters.

The optimum cutoff, which is defined as the value corresponding with the highest accuracy (minimal false-negative and -positive results), was 1.02 mg/L for cystatin C, 77.6 μmol/L for serum creatinine, and 80 mL/min for creatinine clearance. These values are different from those obtained in a previous study of 52 type 2 diabetic patients (5). In that study, the optimum cutoff was 0.93 mg/L for cystatin C, 87.5 μmol/L for serum creatinine, and 75 mL/min for GFR estimated by the Cockroft-Gault method. These differences between studies concerning the values of the greatest diagnostic efficiency of three parameters and their related cutoff levels may be related to the measurement method used. We measured serum cystatin C with a nephelometric immunoassay, which is significantly different from the turbidimetric immunoassay used previously. Moreover, we calculated creatinine clearance from serum and urine

creatinine, which is not done in the Cockcroft-Gault method (5).

Although cystatin C was approximately equivalent to serum creatinine and creatinine clearance in terms of the correlation coefficient and ROC curve, our results showed that serum cystatin C had higher sensitivity and met the criteria for a screening test for early renal failure. Compared to the assay for creatinine clearance, which has a high rate of error due to incomplete urine collection and deficiencies in the assay method, the assay for serum cystatin C is precise and time-saving. Therefore, cystatin C measurement is especially suitable for outpatients. However, it is currently more expensive to measure cystatin C than serum creatinine, so it is not widely used as a GFR marker in this country. For the sensitive prediction of reduced GFR in type 2 diabetic patients, serum cystatin C should be used in combination with serum creatinine and creatinine clearance, especially in patients with a ''blind area'' of serum creatinine values.

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