Use of Serum Cystatin C to Detect Early Decline of Glomerular Filtration Rate in Type 2 Diabetes

Yi-Sun Yang¹, Chiung-Huei Peng², Chih-Kuang Lin³, Chi-Pin Wang⁴ and Chien-Ning Huang¹

Abstract

Object The estimation of serum cystatin C and its practical use for the estimation of the glomerular filtration rate (GFR) in diabetic patients has been previously demonstrated, however, those studies did not use the chronic kidney disease GFR staging. Therefore, we performed this study in type 2 diabetic patients with the aim to examine the usefulness of serum cystatin C to detect early decline of GFR using the staging of chronic kidney disease defined by the National Kidney Foundation.

Methods A total of 102 Taiwanese type 2 diabetic patients were recruited from the Chung-Shan Medical University Hospital. Morning fasting blood and urine samples were obtained for basal metabolic parameters, serum creatinine, serum cystatin C, and albumin-creatinine ratio. GFR was determined by Cockcroft-Gault equation creatinine clearance (CG-CCr).

Results Of the 102 type 2 diabetic patients, 67, 25, and 10 had normo-, micro-, and macroalbuminuria, respectively. Serum cystatin C was superior to serum creatinine in detecting early decline of GFR. The diagnostic accuracy of serum cystatin C was better than serum creatinine for stage 1 and 2 chronic kidney disease (CG-CCr cut-off value of 90 ml/min and 60 ml/min). Furthermore, serum cystatin C was also correlated with urine albumin excretion, which was not true with serum creatinine.

Conclusions These results suggest that serum cystatin C may be an alternative serum marker for the early identification of subjects with a slight reduction of renal function, and also it may be a marker for early glomerular dysfunction in type 2 diabetes.

Key words: Glomerular filtration rate, cystatin C, chronic kidney disease, creatinine, creatinine clearance, urinary albumin excretion

Introduction

Diabetic nephropathy is the single most frequent cause of end-stage renal disease in the world, and is predominantly due to the increasing prevalence of type 2 diabetes mellitus (T2DM). It is characterized by microalbuminuria, subsequent macroalbuminuria, and declining glomerular filtration rate (GFR). An increasing number of type 2 diabetic patients live long enough for nephropathy and end-stage renal disease to develop because of the improvement in the treatment of diabetes, hypertension and coronary heart diseases.

Screening for diabetic nephropathy is currently done by monitoring patients for the development of microalbuminuria, and as adjunct for the estimation of GFR, the determination of serum creatinine (sCr), and creatinine clearance (CCr). Intervention with angiotensin-converting enzyme inhibitor and angiotensin receptor blockers have been shown to delay the development of end-stage renal disease in micro- and macroalbuminuric patients (1). However, there are diabetic patients who have a combination of normo- and microalbuminuria and impaired renal function, but not the traditional decline of GFR with the development of proteinuria (2, 3). In addition, reduced kidney function is associ-
ated with increased incidence of cardiovascular morbidity and mortality in large cohort studies (4, 5). Therefore, it is of worth to develop a more sensitive or specific indicator for detecting early renal impairment in diabetic patients.

GFR can be estimated in several ways. Serum creatinine is the most widely used marker of GFR in the clinical practice, although it is insensitive for early renal disease.

Notably, the sCr level depends on muscle mass and meat intake, and it may have positive interference from glucose, protein and fructose (6, 7). The isotopic and non-isotopic methods for the determination of GFR, though more accurate, are generally expensive, with more complex methodologies involved and are usually impractical for routine use (8, 9). The other common method used is the CCr, a test that compared sCr with creatinine concentrations in a 24-hour urine collection. This procedure is useful, but not as precise. A recently published guideline from the National Kidney Foundation recommended that GFR is estimated from prediction equations taking into account the sCr concentration and some of the following variables: age, gender, and body size, calculated by formulas such as Cockcroft-Gault equation (10, 12).

When renal function decreases, the serum concentration of many low-molecular weight proteins increases. As a consequence, the blood levels of some small proteins, such as lysozyme, β2-microglobulin, and cystatin C, have been proposed as indices of renal function (13, 14). Cystatin C is a low molecular weight protein (13kd) produced by all nucleated human cells, with a stable production rate. It is freely filtered by the glomerulus and catabolized primarily by proximal tubular cells. It is not influenced by renal factors, such as inflammatory, infectious, and liver diseases, or by dietary or constitutional factors that could influence the production rate (15). Cystatin C seems to be a promising candidate as a novel marker of the GFR (3, 16). The Food and Drug Association (FDA) has approved Dade-Behring’s automated immunoassay for this marker. It has been suggested that cystatin C could be especially useful in the detection of early nephropathy, as demonstrated by the increased cystatin C level in patients with hypertension, and microalbuminuria, but with a normal GFR (3). Although there is growing data and literature supporting the use of cystatin C, it is yet not widely used, probably because of some controversial data, lack of references, and lack of standardized laboratory measurement. The aim of the current study was to compare serum cystatin C and sCr as markers for early decline of GFR in T2DM, using the staging of chronic kidney disease (CKD) defined by the National Kidney Foundation and to explore the relationship of urine albumin, GFR, the sCr and cystatin C concentrations (10).

**Methods**

The study was performed on 102 Taiwanese type 2 diabetic patients recruited from the outpatient diabetes clinic of Chung-Shan Medical University Hospital. All patients were under steady-state condition to be included in this study. Steady-state was defined as the lack of a clinical event or diabetes imbalance during at least the past 6 months.

Therapeutic modalities applied included diet, oral hypoglycemic agents or insulin therapy.

The patients were aged from 30 to 80, and 52 were males. Patients with sCr > 1.4 mg/dL, and other known renal diseases and/or factors that influence the urine creatinine and urine albumin are excluded. These patients were recruited in the absence of prior determination of GFR.

Morning and fasting blood and urine samples for measurement of biochemical parameters, serum cystatin C, urine albumin and creatinine were obtained. The albumin: creatinine ratio (ACR) was calculated. Serum cystatin C was measured by an optimized enhanced immunonephrometry method (by the Dade Behring prospec). CCr was calculated according to the Cockcroft-Gault formula (CG-CCr). The CG-CCr formula was standardized for a 1.73 m² body surface area. All biochemical examinations were done centrally and the assays were unchanged during the study period. Serum and urinary creatinine and urinary albumin were measured using a specific enzymatic assay. The patients were divided into those with normo-, micro-, and macroalbuminuria (ACR < 30, 30-299, and ≥ 300 μg/mg creatinine, respectively). They were also divided into 3 groups according to the classification of CKD according to their CG-CCr, stages 1, 2, and 3 (≥ 90, 89-60, and 59-30 ml/min, respectively). Informed consent was obtained from all patients.

**Statistical Analysis**

Statistical analyses were performed using Statistical Package for Social Science programs (SPSS Inc). Spearman’s rank correlation was used for univariate analyses. The groups were compared using analysis of variance and t test. To assess the diagnostic value of the parameters, receiver operating characteristic (ROC) and the area under the curve (AUC) were calculated.

**Results**

Of the 102 patients with type 2 diabetes, 65.7% had normoalbuminuria, 24.2% had microalbuminuria, and 10.1% had macroalbuminuria; 34.3% in CKD stage 1, 40.4% stage 2, and 25.3% stage 3. The demographic and biochemistry characteristics of these patients are shown in Table 1.

The mean value of the sCr significantly differed between females and males (1.07 ± 0.18, 0.88 ± 0.17, respectively, p < 0.01), while no difference was observed for cystatin C values (0.93 ± 0.23, 0.83 ± 0.21, respectively, p = 0.05). Analysis of the data on the basis of age (by stratifying into every two decades:age of 30-50, 50-70, and 70-90), the mean value of the sCr increase with increase of age (0.94 ± 0.2, 0.96 ± 0.2, 1.11 ± 0.2, p < 0.01), while no difference was noted for cystatin C (0.83 ± 0.2, 0.88 ± 0.2, 1.1 ± 0.2, p=0.06). Assessing the influence of body size on the values
of cystatin C and sCr, for body mass index < 20, 20-25 and > 25 kg/m²; the mean value of cystatin C was 0.86 ± 0.28, 0.90 ± 0.20, and 0.91 ± 0.23 (p = 0.73), and 0.97 ± 0.17, 0.97 ± 0.20, and 1.12 ± 0.21 for sCr, respectively (p = 0.02). The mean sCr was 0.88 ± 0.22, range 0.5-1.4. The mean serum cystatin C was 0.97 ± 0.21, range 0.4-1.4. The mean CG-CCR was 83.01 ± 33.9, range 35-228. The mean ACR was 154 ± 945, range 3.1-9540. As shown in Table 2, serum cystatin C concentration increased significantly in patients from normo- to macro- and micro- to macroalbuminuria (all p < 0.001). It was also significantly increased in patients with stage 1 to 3 and stage 2 to 3 groups in CKD classification (all p < 0.001). The sCr also revealed significance, but only in micro- to macroalbuminuria group and CKD stage 2 to stage 3 groups.

A statistically significant correlation was found in CG-CCR, sCr and serum cystatin C (Table 3). A positive correlation between serum cystatin C levels and ACR was also noted. This correlation was not found with sCr. ROC curve analysis for all patients included in the study showed that serum cystatin C had significantly higher diagnostic accuracy than sCr (Figs. 1A, 1B). With a cut-off value of CG-CCR at 90 ml/min, the AUC was 0.846 for serum cystatin C, and 0.757 for sCr (p < 0.05). In discriminating ACR, while with a cut-off value of 300 μg/mg creatinine, the AUC were 0.68 for serum cystatin C, 0.43 for sCr, and 0.4 for CG-CCR (p < 0.05).

**Discussion**

In recent years, several studies have confirmed the usefulness of cystatin C determinations as a marker of early deterioration of GFR, being more sensitive than sCr (17-19). The serum cystatin C level has been proven to be independent of gender, weight, infections, dietary factors and liver diseases, but some suggest it is related to age, and is slightly higher in males (20). Results from Finney et al indicated that the mean value of cystatin C for women was lower, and the difference was smaller than those for creatinine (21). Our results on the basis of gender and age showed consistency with previous studies. As for sCr in our study, the mean concentration was higher in females with statistical significance. Thus, we can confirm that in our patients, cystatin C is independent of gender, age and body size.

In diabetic patients, previous studies have shown that serum cystatin C is more sensitive than sCr for the detection of GFR. Harmoinen et al and Mussap et al showed that serum cystatin C is more sensitive than sCr for the estimation
Table 3. Spearman correlation coefficients of sCr, serum cystatin C, CG-CCr, and ACR

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>sCr</th>
<th>CG-CCr</th>
<th>ACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>.546 (p&lt;0.0001)</td>
<td>-.717 (p&lt;0.0001)</td>
<td>.170 (p=0.05)</td>
</tr>
<tr>
<td>sCr</td>
<td></td>
<td>-.650 (p&lt;0.0001)</td>
<td>-.086 (p=0.29)</td>
</tr>
</tbody>
</table>

sCr, serum creatinine; CG-CCr, Cockcroft-Gault creatinine clearance; ACR, albumin-creatinine ratio

Figure 1A. Non-parametric ROC plots for the diagnostic accuracy of cystatin C, and sCr in distinguishing between CKD stage 2-3, CG-CCr 60 ml/min. Cystatin C area = 0.8498 ± 0.058, p < 0.001; sCr area = 0.757 ± 0.057, p < 0.008.

Figure 1B. Non-parametric ROC plots for the diagnostic accuracy of cystatin C, and sCr in distinguishing between CKD stage 1-2, CG-CCr 90 ml/min. Cystatin C area = 0.846 ± 0.04, p < 0.0001; sCr area = 0.671 ± 0.05, p < 0.001.

Shimizu et al showed a significant relationship between serum cystatin C levels and the prognostic stage in patients with type 2 diabetic nephropathy (26). Bicik et al showed that sensitivity of serum cystatin C is higher in microalbuminuric patients with diabetes (27). On the other hand, Ododo et al, demonstrated that sCr and serum cystatin C is equal in diagnostic accuracy in microalbuminuric and proteinuric diabetic patients (28). These studies differ from each
other because of the use of different methods of GFR measurements, with different cut-off levels of GFR (80, 68 or 60 ml/min) and different methods of sCr and serum cystatin C measurement.

Therefore, we studied the level of serum cystatin C using the FDA approved method in diabetic patients and analyzed the results in view of the markers frequently and routinely used in clinical practice, such as ACR, sCr, and calculated CG-CCr. In addition, the American Diabetes Association applied the CKD staging to diabetic nephropathy (29).

This classification was not used in the previous studies of cystatin C in diabetic nephropathy.

The present results showed similar results compared to the previous studies, despite the changes in GFR classification. Both serum cystatin C and sCr revealed a significant difference in the ACR and CKD staging group. However, in the ACR group, the mean value of the cystatin C was significantly higher in the microalbuminuric group compared to normoalbuminuric group. However, sCr also did not show significance. In the CKD staging group, the mean value of cystatin C was significantly higher in stage 1 compared to stage 2 group. While sCr again did not show this significance. Therefore, while serum cystatin C has been thought to be a better marker to detect early nephropathy, and the early decline of GFR it was confirmed in this study.

Both cystatin C and sCr were better to predict GFR decline than appearance of albuminuria. However, we found a positive correlation between serum cystatin C levels and ACR. This correlation was not found with sCr. Therefore, we suggest that serum cystatin C could also reflect glomerular dysfunction.

From the area under the ROC curve we found that the diagnostic accuracy of serum cystatin C is considerably better than that of sCr in discriminating CKD stages 1, 2 and 3. The area under the ROC curve in our study was not as good as in previous studies when discriminating the GFR at a cut-off level of 60 ml/min (22-25). However, using an increased cut-off value of 90 ml/min, the present results indicated that serum cystatin C is also a good indicator of early decline of GFR and CKD stage 1 to 2, compared with the previous studies.

The present study did not use isotopic or non-isotopic methods for the determination of GFR. However, the superiority of cystatin C over sCr with respect to GFR measurement has been confirmed by both superior correlation coefficients and greater ROC-plot AUC values in a recent meta-analysis (30). In this large study, they used CCr as a GFR marker, because there was no gold standard for comparing the diagnostic accuracy of cystatin C and sCr. Our results also demonstrated a superior correlation of cystatin C over sCr with respect to GFR measurement. However, this study was conducted cross-sectionally.

Further follow-up of renal function parameter changes for a more accurate estimation of GFR is needed for type 2 diabetic patients. Recently, Perkins et al showed that serial measurements of serum cystatin C could accurately detect trends in renal function in patients with normal or elevated GFR (31). Thus, it is noteworthy to further study serum cystatin C in CKD.

In conclusion, serum cystatin C may be used as an alternative to creatinine and creatinine clearance to screen for and monitor kidney function in diabetic patients. It may be especially useful in detecting early decreased renal function, and in those for which creatinine or urine albumin measurement is a problem. In addition, serum cystatin C also may be a marker for glomerular dysfunction in type 2 diabetic nephropathy.

This research was supported by Chung-Shan Medical University.

References

14. Simonsen O, Grubb A, Thyliss H. The blood serum concentration

© 2007 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imindex.html