Serum Cystatin C Concentration as a Marker of Glomerular Filtration Rate in Patients with Various Renal Diseases

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Abstract

Objective The present study was undertaken to evaluate clinical application of serum cystatin C as a new marker of glomerular filtration rate (GFR) in patients with various renal diseases.

Patients and Methods A total of 140 patients were enrolled in the study. We measured the serum cystatin C levels and compared them with creatinine clearance (Ccr) and inulin clearance (Cin) as an indicator of GFR.

Results There was a significant positive correlation between serum cystatin C and creatinine levels (r=0.928). Serum cystatin C was inversely correlated with creatinine clearance. Moreover, the reciprocal serum cystatin C level was positively correlated with Cin (r=0.882). The receiver-operating characteristic curve of serum cystatin C and creatinine demonstrated that the diagnostic accuracy of the serum cystatin C level is superior to that of creatinine in identifying individuals with reduced GFR.

Conclusion These results indicated that measurement of serum cystatin C is useful to estimate GFR, and in particular, to detect a mild reduction of GFR in patients with renal diseases.

Key words: creatinine, inulin, glomerulonephritis

Introduction

Glomerular filtration rate (GFR) is an important measurement in nephrology as a functional parameter. Serum creatinine and urea levels and creatinine clearance (Ccr) are currently used in the assessment of GFR. Previous reports, however, have emphasized the effects of differences among individuals in the rates of tubular secretion and total renal excretion of creatinine (1, 2). This hampers the reliability of serum creatinine as a marker for GFR. Since the tubular secretion of creatinine increases as the GFR decreases, serum creatinine systematically underestimates the severity of the renal disease (3). Moreover, a variety of non-renal events may influence the serum creatinine levels determined. Drugs, like trimethoprim-sulfa and cimetidine, compete with creatinine related to tubular secretion (4) and cause interference in some procedures for creatinine determination resulting in spurious high or low creatinine values. Therefore, it is necessary to determine a serum marker able to detect renal function impairment, especially at the initial phase.

Cystatin C is a non-glycosylated 13 kD basic protein that is produced by all investigated nucleated cells, with a stable production rate (5). 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. Several recent studies have suggested that serum cystatin C measurement correlates with GFR (6, 7). A few recent reports clearly indicate that serum cystatin C is superior to serum creatinine as a marker for GFR in adults (8-11) and children (12).

The present study was undertaken to evaluate a rapid and automated procedure for the quantification of serum cystatin C levels by using particle-enhanced immunonephelometry, newly developed by Daido Behring Co., in patients with various renal diseases. We then compared serum cystatin C level to serum creatinine level and the clearance of inulin and creatinine as markers of GFR and analyzed their changes according to renal failure.

Patients and Methods

Patients

The study consisted of 140 patients (72 males and 68 females) ranging in age from 20 to 68 (average age 42 years old). The underlying renal diseases were chronic glomerulonephritis (n=39), IgA nephropathy (n=24), diabetic nephropathy (n=14), hypertensive nephrosclerosis (n=18), polycystic kidney disease (n=15), chronic pyelonephritis (n=6), lupus nephritis (n=16), and Henoch-Schoenlein purpura nephritis (n=8). Patients were divided into 3 groups according to creati-
nine clearance, an indicator of GFR. Group 1 consisted of patients with a normal GFR (70 ml/min < Ccr), group 2 consisted of patients with a mild reduction of GFR (30 < Ccr ≤ 70 ml/min), and group 3 consisted of patients with a remarkable reduction of GFR (Ccr ≤ 30 ml/min). Blood was collected from all patients to measure cystatin C, inulin, and creatinine levels. Informed consent was obtained from each patient. Serum cystatin C levels of 72 healthy volunteers (36 males and 36 females, mean age; 40.0±12.5 years) were also assessed.

Measurement of serum cystatin C and creatinine
As previously described (13), serum cystatin C levels were determined by particle-enhanced immunonephelometry on the Behring nephrometer system (Dade Behring Co., Tokyo) based on the report by Finney et al (14). Serum creatinine was determined by the TOSHIBA TBA-80FR system using the AUTOSERA CRE Kit (Daichi Chemical Co., Tokyo). A standard clearance technique was used for measurement for 2-hr intrinsic creatinine clearance (Ccr).

Assessment of inulin clearance
The inulin clearance study included 26 patients (17 males and 9 females) ranging in age from 19 to 67 (average age 41.0 years old). The underlying renal diseases were IgA nephropathy in 10 patients, focal glomerulosclerosis in 8 patients, membranous nephropathy in 3 patients, Henoch-Schoenlein purpura nephritis in 2 patients, lupus nephritis in 1 patients, minimal change nephrotic syndrome in 1 patient and hypertensive nephrosclerosis in 1 patient. A standard clearance technique was used for measurement of inulin clearance (Cin). After a prime dose of inulin (Inutest; 25%, Laevosan Gesellschaft, Vienna, Austria) 64 mg/kg body weight, a continuous intravenous infusion of 1–2 mg/kg per min inulin was given. Urine samples were collected by spontaneous micturition at 30-min intervals, and blood samples were drawn midway between each urine collection period. The baseline GFR corresponds to the mean values of the three periods.

Statistical analysis
All data are expressed as mean±SE. The significance of differences between groups was determined by using Student t-test or Mann-Whitney U test and the P value <0.05 was considered significant. Pearson’s correlation analysis was used to observe the relationship between serum cystatin C concentration and other clinical parameters. Sensitivity and specificity of serum cystatin C and creatinine were assessed by receiver-operating characteristic (ROC) curves according to the procedure of Hanley and McNeil (15).

Results

Characteristics of serum cystatin C in the normal population
The upper cutoff values of serum cystatin C and creatinine in our laboratory were 1.0 mg/l and 1.2 mg/dl, respectively. The cystatin C concentration in serum was stable for at least 2 days when stored at room temperature, for up to 1 weeks at 4°C, at least 1 week at −20°C. There was a significant increase in the average serum cystatin C levels in males (0.815±0.019 mg/l) compared with those in females (0.650±0.019 mg/l) in the normal population.

Comparison of serum levels of cystatin C and creatinine in patients with renal diseases
The serum cystatin C levels in the patients ranged from 0.4 to 4.3 mg/l, and serum creatinine levels ranged from 0.5 to 6.7 mg/dl. Mean values of serum cystatin C and creatinine for different ranges of Ccr are shown in Table 1. Serum cystatin C increased in accordance with the reduction of renal function in patients with various renal diseases. To assess the question of whether or not the age of the subject reflects serum cystatin C values, the relationship between age and serum cystatin C was evaluated. No significant correlation was found between age and the serum cystatin C level.

There was a significant positive correlation between serum cystatin C and creatinine levels (r=0.941, cystatin C=1.335×creatinine−0.18). These findings support the concept that cystatin C and creatinine have similar properties as serum markers of renal function.

Relationship between serum levels of cystatin C and creatinine and other GFR markers
The relationship between serum levels of cystatin C and Ccr is shown in Fig. 1, indicating the classical curvilinear relationship demonstrated by serum creatinine. Correlation between the reciprocal serum cystatin C levels and Ccr (r=0.872) was similar to that between the reciprocal serum creatinine levels.

Table 1. Mean Values of Serum Cystatin C and Creatinine in Different Ranges of Creatinine Clearance

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Serum cystatin C (mg/l)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Creatinine clearance (Ccr) (ml/min/1.48 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccr&gt;70</td>
<td>Male (36)</td>
<td>0.82±0.02</td>
<td>0.98±0.16</td>
<td>93.99±18.04</td>
</tr>
<tr>
<td></td>
<td>Female (52)</td>
<td>0.69±0.12</td>
<td>0.74±0.11</td>
<td>90.25±16.01</td>
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<tr>
<td>30&lt;Ccr ≤ 70</td>
<td>Male (26)</td>
<td>1.42±0.39</td>
<td>1.68±0.50</td>
<td>48.76±12.58</td>
</tr>
<tr>
<td></td>
<td>Female (22)</td>
<td>1.06±0.32</td>
<td>1.05±0.29</td>
<td>55.28±11.37</td>
</tr>
<tr>
<td>Ccr ≤ 30</td>
<td>Male (12)</td>
<td>2.92±0.70</td>
<td>4.11±1.17</td>
<td>18.17±6.01</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>2.30±0.18</td>
<td>2.05±0.24</td>
<td>25.00±3.46</td>
</tr>
</tbody>
</table>

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![Graph showing correlation between serum levels of cystatin C and creatinine and creatinine clearance in patients with various renal diseases. n=140.](image)

Figure 1. Correlation between serum levels of cystatin C and creatinine and creatinine clearance in patients with various renal diseases. n=140.

and Cr (r=0.839). Table 2 shows the comparison of the correlation coefficient between Cr and reciprocal serum levels of cystatin C and creatinine.

Since Cn is thought to be a more accurate marker for GFR than Cr, we determined the correlation between serum cystatin C levels and Cn in the patients studied. As shown in Fig. 2A, the reciprocal serum cystatin C concentration was positively correlated with Cn (r=0.870, 1/cystatin C=0.432xCn+0.009). There was also positive correlation between the reciprocal serum creatinine concentration and Cn (r=0.657, 1/creatinine=0.49xCn+0.007) (Fig. 2B).

**ROC analysis for serum levels of cystatin C and creatinine**

We chose a cutoff value of 70 ml/min/1.48 m² for the definition of renal failure. To assess the diagnostic accuracy of the serum levels of cystatin C and creatinine in predicting reduced Cr, we conducted the ROC plots. We estimated that significant, with a type 1 error rate, could be taken to be 0.05 (two-tailed) and a power of 80% as previously described (16). As shown in Fig. 3, the area under the serum cystatin C curve was significantly larger than those under the creatinine curve (p<0.05), demonstrating that the diagnostic accuracy of the serum cystatin C level is superior to that of creatinine in identifying individuals with reduced Cr.

**Discussion**

An alternative low molecular weight protein that has been proposed as a marker of GFR is cystatin C, which is a nonglycosylated basic protein of cysteine proteinase inhibitors; it is produced by all investigated nucleated cells and its production rate is unaltered in inflammatory conditions. The measurement of cystatin C and its practical use in estimating GFR have been studied by several investigators (6-12). However, the wide variation of reference intervals reflects the difficulties of standardizing an immunological method. The correlation between turbidimetric and nephelometric methods has been shown to be good, albeit early reports indicated a significant bias. This was thought to be due to the differences in assignment of values to the calibrators, and a reduced bias has been reported more recently. In 1994-95, two fully automated latex particle-enhanced turbidimetry assays for cystatin C (9, 10) were developed. These assays were both rapid, automated methods for measuring cystatin C. Accordingly, we tested a cystatin C measurement kit by particle-enhanced immunonephelometry on the Behring nephelometer system (16) sup-

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**Table 2. Comparison of the Correlation Coefficient between Creatinine Clearance (Cr) and Reciprocal Serum Cystatin C and Creatinine**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>1/cystatin C</th>
<th>1/creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cn&gt;70</td>
<td>Male (36)</td>
<td>0.113</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>Female (52)</td>
<td>0.142</td>
<td>0.456</td>
</tr>
<tr>
<td>30&lt;Cr&lt;70</td>
<td>Male (26)</td>
<td>0.509</td>
<td>0.563</td>
</tr>
<tr>
<td></td>
<td>Female (22)</td>
<td>0.563</td>
<td>0.391</td>
</tr>
<tr>
<td>Cr&lt;30</td>
<td>Male (12)</td>
<td>0.840</td>
<td>0.722</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>0.785</td>
<td>0.175</td>
</tr>
<tr>
<td>60&lt;Cr&lt;80</td>
<td>Male (14)</td>
<td>0.257</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Female (26)</td>
<td>0.255</td>
<td>0.161</td>
</tr>
</tbody>
</table>
The present study has demonstrated that measurement of the serum cystatin C level could be useful to evaluate GFR in patients with impaired renal function due to various renal diseases. There was a significant positive correlation between serum cystatin C and creatinine levels. To evaluate the diagnostic accuracy of the serum levels of cystatin C and creatinine, we constructed ROC plots and found that the diagnostic accuracy of the serum cystatin C was superior to that of creatinine in the patients studied. A mild reduction of GFR was detected more easily by serum cystatin C than by serum creatinine since the upper normal range is 1.0 mg/l, corresponding to that of serum creatinine, 1.2 mg/dl. No age-dependence of the serum level of serum cystatin C could be demonstrated in our patients with various renal diseases as previously described by Bokenkamp et al (17).

If serum cystatin C is to replace serum creatinine or creatinine clearance as the routine method of choice, it is important to determine the correlation of results with those using a reference method. Newman et al (10) in studying 206 patients with various types of renal disease demonstrated that the increase in cystatin C occurred earlier than with creatinine as the GFR value fell, using the 51Cr-EDTA single injection technique as the reference method. Tian et al (11) similarly found that a mild reduction in GFR was more readily detected by a change in cystatin C than in creatinine. In the current study, we have shown that the serum cystatin C concentration is well correlated with Cin, an indicator for GFR, in patients with various types of renal diseases. Recent reports (18, 19), however, showed that plasma cystatin C measurement appears to be broadly equivalent to serum creatinine measurement for estimation of GFR in pediatric patients when compared with Cin.

This discrepancy may be due to the gender of population studied. It is suggested that the combined testing of cystatin C and creatinine might be used as a check for the validity of the data, given the high overall degree of correlation between serum crea-
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tinine and Ccr.

In conclusion, the cystatin C assay by particle-enhanced immunonephelometry was found to be a sensitive, fully automated, and rapid method, essential for the quick turnaround necessary in a routine hospital laboratory. Measurement of serum cystatin C is useful to estimate GFR, especially to detect a mild reduction of GFR in patients with various renal diseases. Further prospective studies are necessary to monitor serum cystatin C concentration in patients with different stages of renal diseases to assess cystatin C as a potentially more sensitive and specific marker of GFR than creatinine.

Acknowledgements: We thank Daido Behring Co. for providing us kits for automated measurement of cystatin C. This was in part presented at the 98th Annual Meeting of Japanese Society of Internal Medicine, on April 13 in 2001.

References