Original Article

Serum Cystatin C Level Is a Marker of End-Organ Damage in Patients with Essential Hypertension

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High urinary albumin excretion rate (AER) has been associated with the presence of atherosclerotic vascular damages and is an independent risk factor for all causes of death and cardiovascular morbidity and mortality in essential hypertensive patients. Serum cystatin C (s-CC) is a recently identified nonglycosylated 13-kD basic protein that has been suggested to be a useful marker of glomerular filtration rate. In the present study, we investigated the relationship between s-CC level and end-organ damages in the kidney, heart, and vessels of patients with essential hypertension. Sixty patients with essential hypertension participated in the present study. Patients with renal failure were excluded. Serum-CC level was measured by a particle-enhanced turbidimetric assay. Left ventricular mass index (LVMI) and intima media thickness (IMT) in the common carotid arteries were evaluated by ultrasound images. Twenty-four-hour blood pressure was measured by a cuff-oscillometric method. Serum-CC level was negatively correlated with creatinine clearance ($r = -0.617$, $p < 0.0001$). It was also correlated with mean 24-h systolic blood pressure (24h-SBP) ($r = 0.308$, $p = 0.0167$), LVMI ($r = 0.528$, $p < 0.0001$), and IMT ($r = 0.539$, $p < 0.0001$). Both AER and s-CC level were independently associated with mean 24h-SBP. AER but not s-CC level was associated with HDL-cholesterol. The present study was the first to demonstrate that s-CC level is a useful and convenient parameter of renal function, and may also prove to be an early marker of the severity of end-organ damage in patients with essential hypertension. (Hypertens Res 2003; 26: 895–899)

Key Words: cystatin C, hypertension, albuminuria, carotid atherosclerosis, left ventricular hypertrophy

Introduction

Essential hypertension is a major risk factor for the progression of cardiovascular damages. In particular, microalbuminuria is an independent risk factor for all causes of death and cardiovascular morbidity and mortality in essential hypertensive patients (1–3). Increased albuminuria is thought to be associated with the presence of early signs of hypertensive and atherosclerotic vascular damages, such as increased carotid wall thickness (4, 5), retinal vascular changes (6, 7), and left ventricular hypertrophy (LVH) (8). Measurement of the urinary albumin excretion rate (AER) is thus indispensable for hypertensive subjects. Currently, however, AER must be measured by 24-h urine collection, a cumbersome method which can lead to poor reproducibility.

Cystatin C, a nonglycosylated 13-kD basic protein, is a member of the cystatin superfamily of endogenous cysteine proteinase inhibitors (9). It is produced by all nucleated cells, and its production rate is not affected under inflammatory conditions (10–12). Cystatin C is freely filtered by the glomerulus, and is almost completely reabsorbed and catabolized in the tubules (13). Based on these characteristics, the serum cystatin C (s-CC) level is mainly determined by the glomerular filtration rate (GFR) for individuals (10–13), and can be easily and reproducibly measured by a blood sample.
As well as by serum creatinine concentration.

In the present study, we investigated the relationship between s-CC level and end-organ damages, and demonstrated, for the first time, an association between s-CC level and other well-known markers of end-organ damages, such as AER, LVH, and carotid artery wall thickness, in patients with essential hypertension.

Methods

Patients

Study patients with essential hypertension participated in this study. They were recruited from among consecutive cases admitted to Ehime University Hospital from July 1997 to July 2001. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg as measured three times in the sitting position using a brachial sphygmomanometer. Patients with renal damage, which was defined as serum creatinine of more than 1.5 mg/dl, were excluded. Patients with diabetes mellitus were also excluded. All patients were untreated or had discontinued therapy at least 2 weeks before the investigation. All patients gave their informed consent to participate in the procedures.

Blood and Urine Sampling

Serum-CC level was measured by a particle-enhanced turbidimetric assay (Cystatin C PET Kit; DACO, Glostrup, Denmark). Measurement of creatinine, albumin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride concentrations was carried out using an automatic analyzer (model TBA-60S; Toshiba Inc., Tokyo, Japan). Each patient collected his or her own urine for 24 h for the determination of AER and creatinine clearance (Ccr). Ccr was standardized by the body surface area, and was used as an index of GFR.

Determination of Left Ventricular Mass Index

Echocardiographic studies were carried out using an SSD-5500 echocardiograph with a 3.5-MHz transducer (Aloka Inc., Tokyo, Japan) according to the recommendations of the American Society of Echocardiography (14). Left ventricular mass (LVM) was estimated using the formula of Devereux and Reichek (15), and was corrected by the body surface area to obtain the LVM index (LVMI).

Ultrasound Analysis of the Common Carotid Arteries

The carotid arteries were evaluated using an SSD-2000 transducer scanner (Aloka Inc.) employing a 7.5-MHz probe as described previously (16). After the subject had rested for at least 10 min in the supine position with his or her neck in slight hyperextension, we examined the optimal visualization of the common carotid artery (CCA), carotid bulb, and extracranial internal and external carotid arteries on both sides. The intima media thickness (IMT) of the far wall was measured in the CCA at both 1 and 2 cm proximal to the bulb from the anterior, lateral, and posterior approaches, and then averaged to obtain the mean IMT. The level of discrete plaque was not measured.

Twenty Four-Hour Blood Pressure Determination

Twenty four-hour blood pressure (24h-BP) was measured by a cuff-oscillometric method using an FB-250 oscillometer (Fukuda Denshi Co., Ltd., Tokyo, Japan). Blood pressure (BP) was measured every 30 min from 6:00 AM to 10:00 PM and every 60 min from 10:00 PM to 6:00 AM of the following day (17).

Statistical Analysis

All values are expressed as the means ± SD. Pearson’s correlation coefficient was used to assess the association. A stepwise regression analysis was used to evaluate the association between each of several factors and either AER or s-CC level. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Table 1 shows the clinical characteristics of the subjects. The mean value of the s-CC levels was 0.84 ± 0.20 mg/l. We also measured s-CC in 98 healthy subjects; the mean value...
(0.44 ± 0.34 mg/l) was substantially lower than that in hypertensive subjects. The intra- and inter-assay variabilities of the method used in our laboratory were 6.2% and 8.6%, respectively. The correlation between s-CC and Ccr ($r = -0.617, p < 0.0001$) was much higher than that between serum creatinine and Ccr ($r = 0.348, p = 0.0064$) (Fig. 1A). The significant correlation between s-CC level and Ccr was seen in both age groups (< 65 years of age: $r = 0.649, p < 0.0001$; > 65 years of age: $r = 0.583, p = 0.0056$). Serum-CC level was positively correlated with AER (Fig. 1B). As shown in Fig. 2A, s-CC level was associated with mean 24h-SBP, but not with mean 24h-DBP ($r = 0.194, p = 0.1601$). The s-CC level was also associated with LVMI and IMT (Fig. 2B,C).

A stepwise regression analysis for AER or s-CC level was performed with age, body mass index, total cholesterol, HDL cholesterol, and mean 24h-SBP as independent variables (Table 2). AER and s-CC level were independently associated with mean 24h-SBP, whereas AER but not s-CC level was associated with HDL-cholesterol.

**Discussion**

Renal function is thought to be a key determinant factor for the outcome of cardiovascular diseases (18). Renal function is usually evaluated by estimating the GFR by means of Ccr, or more conveniently by measuring serum creatinine concentration. However, serum creatinine concentration is increased only when the GFR is reduced by approximately 50%. Recently, s-CC level has been reported to be a more sensitive marker of the GFR compared with serum creatinine concentration, particularly for individuals with a small-to-moderate decrease in the GFR to a value within the so-called creatinine-blind GFR range (13, 19). In the present study, we established the clinical efficacy of measuring the s-CC level as an early premature marker of the GFR, and demonstrated that the s-CC level is correlated with Ccr and AER in patients with essential hypertension (Fig. 1).

Microalbuminuria indicates the presence of microvascular damages and can be considered predictive for the development of overt proteinuria and for cardiovascular mortality independent of the presence of other risk factors (1–3). AER is thought to be associated with LVM, as well as with IMT of
Table 2. Stepwise Regression Analysis for Urinary Albumin Excretion Rate and Cystatin C

<table>
<thead>
<tr>
<th></th>
<th>Partial correlation coefficient</th>
<th>p value</th>
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<tbody>
<tr>
<td>Albumin excretion rate</td>
<td></td>
<td></td>
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<tr>
<td>Mean 24-h systolic blood pressure</td>
<td>0.389</td>
<td>0.0014</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
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<td>0.0220</td>
</tr>
<tr>
<td>Cystatin C</td>
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<td></td>
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<tr>
<td>Age</td>
<td>0.316</td>
<td>0.0115</td>
</tr>
<tr>
<td>Mean 24-h systolic blood pressure</td>
<td>0.252</td>
<td>0.0414</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein.

tabolized by the tubular cells. In essential hypertension, microalbuminuria is not induced by a defect of tubular reabsorption, but rather by a small increase in glomerular vascular permeability followed by glomerular injury and/or an increase in the intra-glomerular pressure (29, 30). These observations suggest that the s-CC level is a pure marker for the GFR, and will eventually become a sensitive marker in the early stage of renal dysfunction, especially in patients with essential hypertension.

Since AER is determined by 24-h urine collections, it is influenced by many physiological conditions, including physical activity, body position, and acute illness with fever (31). In contrast, s-CC is constantly produced by all nucleated cells, its level is unchanged under inflammatory conditions (10–12), and it can be easily measured by a single blood sampling. Even during pregnancy, as reported by Strevens et al., the s-CC is a reliable marker of GFR (32).

Recently, β2 microglobulin was used clinically as an additional serum parameter of GFR. However, β2 microglobulin is increased in patients with malignant disease, collagen disease, and infectious disease (33). Furthermore, Tamba et al. reported that serum β2 microglobulin had a significantly lower sensitivity than s-CC for detecting mild reductions of GFR (34). Therefore, the s-CC level is thought to be a stable and convenient parameter of renal function for individuals. Recent reports have shown that s-CC is increased in patients with asthma, particularly when treated with methylpredonisolone, or in patients with malignant diseases such as melanoma and colorectal cancer (35, 36). Further studies will therefore be needed to clarify the factors that influence the s-CC level.

In conclusion, the present study was the first to report that measurement of the s-CC level is of clinical value for the management of patients with essential hypertension, and that the s-CC level is also a clinically useful and sensitive marker of the severity of end-organ damages in patients with essential hypertension.

References

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