Serum Cystatin C May Predict the Early Prognostic Stages of Patients With Type 2 Diabetic Nephropathy

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We determined the relationship between levels of serum cystatin C or serum creatinine (s-Cr) and prognostic stages of type 2 diabetic nephropathy. Serum samples from 174 patients with type 2 diabetes were obtained from Juntendo University Hospital, Tokyo and Juntendo Urayasu Hospital, Chiba, Japan. They were classified into four groups according to the Report of the Ministry of Health and Welfare of Japan as follows: Stage I (normoalbuminuric stage), Stage II (microalbuminuric stage), Stage IIIA (macroalbuminuric stage without renal dysfunction), Stage IIIB (macroalbuminuric stage with renal dysfunction), and Stage IV (renal failure stage). Among these patients, 68 were Stage I, 29 Stage II, 32 Stage IIIA, 17 Stage IIIB, and 28 Stage IV. The levels of serum cystatin C were measured using the Dade Behring Cystatin C assay with automated Dade Behring Nephelometer II (BNII) (Dade Behring Marburg GmbH, Germany). The mean levels of serum cystatin C in Stage IIIA were significantly higher than those in Stage I or II ($P < 0.00001$, $P < 0.0005$, respectively). The mean levels of serum cystatin C in Stage IIIB and Stage IV were also significantly higher than those in Stage I ($P < 0.00001$). However, the mean levels of serum creatinine (s-Cr) in Stage IIIA were not significantly higher than those in Stage I or II. The levels of s-Cr in Stage IIIB and Stage IV were significantly higher than those in Stage I ($P < 0.00001$). Receiver operating characteristic (ROC) plots demonstrated that the area under the curve (AUC) of cystatin C (0.76) was greater than that of s-Cr (0.66). As an early prognostic marker of type 2 diabetic nephropathy, serum cystatin C was better than s-Cr in terms of sensitivity and specificity. It appears that the levels of serum cystatin C may predict early prognostic stages of patients with type 2 diabetic nephropathy. J. Clin. Lab. Anal. 17:164–167, 2003. © 2003 Wiley-Liss, Inc.

Key words: serum cystatin C; s-Cr; Ccr; type 2 diabetic nephropathy; GFR

INTRODUCTION

Glomerular filtration rate (GFR) is the best functional parameter in renal diseases. Inulin and 51Cr-EDTA plasma clearances are the standard methods for measuring GFR, although they are difficult to perform, time consuming, and expensive. Serum creatinine (s-Cr) and creatinine clearance (Ccr) are widely used to assess GFR. However, it appears that the levels of Ccr are 20% lower than those of GFR when measured by inulin clearance. s-Cr is influenced by tubular secretion of creatinine and the patient's muscular mass. Cystatin C is a small non-glycosylated 13 KD basic protein of the cystatin superfamily of cysteine proteinase inhibitors, which are produced by all nucleated cells (1). It is freely filtered by the glomerulus, reabsorbed, and catabolized in the tubules. The stable production rate of cystatin C strongly indicated that the GFR is the major determinant of cystatin C levels in sera (2). Newman et al. (3) reported that serum cystatin C has been shown to be, in all likelihood, a more sensitive marker of early deterioration of GFR than s-Cr. Recently, it was reported that serum cystatin C

may provide an early prognostic marker of patients with IgA nephropathy and various chronic glomerular diseases (4,5). The purpose of this study was to determine the relationship between levels of serum cystatin C or s-Cr and prognostic stages of type 2 diabetic nephropathy.

MATERIALS AND METHODS

Serum Samples and Classification of Type 2 Diabetic Nephropathy in Japan

Serum samples from 174 patients with type 2 diabetes were obtained from Juntendo University Hospital, Tokyo and Juntendo Urayasu Hospital, Chiba, Japan. The serum samples were stored at $-20°C$ prior to use.

They were classified into four groups according to the Report of the Ministry of Health and Welfare of Japan as follows (6): Stage I (normoalbuminuric stage), Stage II (microalbuminuric stage), Stage IIIA (macroalbuminuric stage without renal dysfunction), Stage IIIB (macroalbuminuric stage with renal dysfunction), and Stage IV (renal failure stage). Among these patients, 68 were Stage I, 29 Stage II, 32 Stage IIIA, 17 Stage IIIB, and 28 were Stage IV.

Detection of Serum Cystatin C and Creatinine (s-Cr)

The levels of serum cystatin C were measured using the Dade Behring Nephelometer II (BNII software version 2.0) (7). The N Latex Cystatin C kit (Lot No. 29577, Dade Behring Diagnostics, Marburg, Germany) and a fully automated particle-enhanced nephelometric immunoassay were used in this study. The values of cystatin C obtained from 276 healthy controls ranged from 0.50 to 0.86 mg/L (mean $\pm$ SE; 0.66 ± 0.01 mg/L) (8). Levels of serum creatinine (s-Cr) were measured by routine methods in our hospitals. There were no significant changes in the levels of s-Cr between the two hospitals.

Statistical Analysis

The mean levels of cystatin C and creatinine in sera were compared. A value of $P < 0.01$ was regarded as significant. Sensitivity and specificity of s-Cr and serum cystatin C were assessed by receiver operating characteristic (ROC) curves. ROC curves demonstrate the trade-off between sensitivity and specificity at different values for each variable. An ideal diagnostic test should have a cut-off point near the upper left-hand corner of the graph at a point where both sensitivity and specificity are maximized.

RESULTS

Comparison of Mean Levels of Cystatin C and Creatinine in Sera

The mean levels of serum cystatin C and s-Cr in patients with type 2 diabetes are shown in Tables 1 and 2. The mean levels of serum cystatin C in Stage IIIA were significantly higher than those in Stage I or II ($P < 0.00001$, $P < 0.0005$, respectively). The mean levels of serum cystatin C in Stage IIIB and Stage IV were also significantly higher than those in Stage I ($P < 0.00001$). There were no significant changes in the levels of serum cystatin C between Stage I and II (Fig. 1a). However, the mean levels of s-Cr in Stage IIIA were not significantly higher than those in Stage I or II. The levels of s-Cr in Stage IIIB and Stage IV were significantly higher than those in Stage I ($P < 0.00001$). There were no significant changes in the levels of s-Cr between Stage I and II (Fig. 1b).

ROC Curve Analysis

ROC curve analysis was performed to determine if serum cystatin C is useful in predicting the earlier prognostic stage (Stage II) of type 2 diabetic nephropathy. ROC plots demonstrated that the area under the curve (AUC) of cystatin C (0.76) was greater than that of creatinine (0.66) (Fig. 2). To distinguish between Stages II and IIIa, sensitivity and specificity of serum cystatin C were better than those of s-Cr.

DISCUSSION

In recent years, there have been several reports suggesting that serum cystatin C measurement correlates

TABLE 1. Mean levels of serum cystatin C in patients with type 2 diabetic nephropathy

Stage	Number	Mean $\pm$ SD (mg/L)
I	68	0.679 ± 0.096
II	29	0.705 ± 0.118
IIIa	32	0.896 ± 0.242
IIIb	17	1.506 ± 0.339
IV	28	3.049 ± 0.961

TABLE 2. Mean levels of serum creatinine inpatients with type 2 diabetic nephropathy

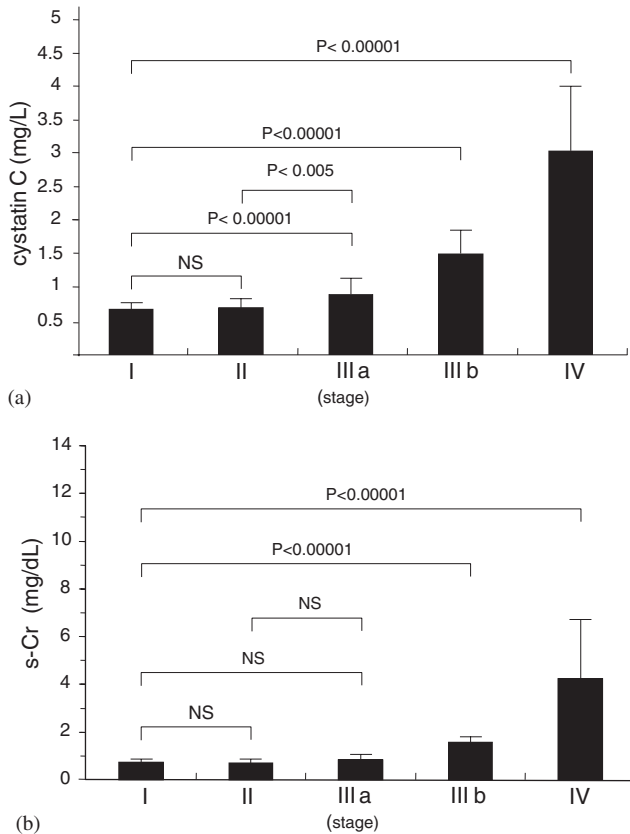| Stage	Number	Mean $\pm$ SD (mg/dl)
I	68	0.723 ± 0.151
II	29	0.722 ± 0.133
IIIa	32	0.823 ± 0.226
IIIb	17	1.562 ± 0.24
IV	28	4.25 ± 2.459

Fig. 1. **a:** Relationship between levels of serum cystatin C and prognostic stages of type 2 diabetic nephropathy. **b:** Relationship between levels of serum creatinine and prognostic stages of type 2 diabetic nephropathy.
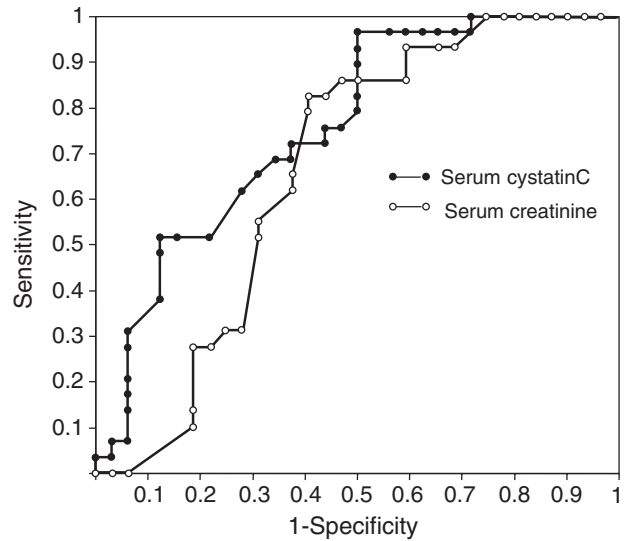

Fig. 2. Nonparametric receiver operating characteristic (ROC) plots to assess the diagnostic efficiency of serum cystatin C and serum creatinine (s-Cr) in distinguishing between Stages II and IIIa.

with the GFR. The authors reported a relationship between levels of serum cystatin C and prognostic stages in patients with IgA nephropathy, which was the most common chronic glomerulonephritis in a multicenter trial in Japan (4). The levels of serum cystatin C were statistically more correlated with the prognostic stage of IgA nephropathy than were the levels of s-Cr and Ccr. In particular, potential utilization of serum cystatin C was observed in the early stage of IgA nephropathy. Recently, the relationship between levels of serum cystatin C or s-Cr and grade of CCr in patients with various chronic glomerular diseases was also determined (5). Ccr levels were classified into six groups according to the Guidelines of the Japanese Society of Nephrology (JSN) as follows (9): Grade 1 (normal renal function); Grade 2 (slight decrease of renal function); Grade 3 (moderate decrease of renal function); Grade 4 (severe decrease of renal function); Grade 5 (renal failure); and Grade 6 (uremia). The mean levels of serum cystatin C in Grade 3 patients were significantly higher than those in Grade 1. The mean levels of serum cystatin C in Grades 4, 5, and 6 were also significantly higher than

those in Grade 1. However, the mean levels of s-Cr in Grade 3 patients were not significantly higher than those in Grade 1. The levels of s-Cr in Grades 4, 5, and 6 were significantly higher than those in Grade 1. The increase of serum cystatin C levels occurred earlier than that of s-Cr in various glomerular diseases. It appears that the levels of serum cystatin C may provide an early prognostic marker for patients with various glomerular diseases superior to s-Cr (5).

The results of this study showed a significant relationship between levels of serum cystatin C and prognostic stages in patients with type 2 diabetic nephropathy. The mean levels of serum cystatin C in Stage IIIA were significantly higher than those in Stage I or II. However, the mean levels of s-Cr in Stage IIIA were not significantly higher than those in Stage I or II. ROC plots demonstrated that the AUC of serum cystatin C (0.76) was greater than that of s-Cr (0.66). Thus, sensitivity and specificity of serum cystatin C were better than those of s-Cr. It appears that the levels of serum cystatin C can predict the early prognostic stage of patients with type 2 diabetic nephropathy.

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