β -trace Protein, a New Marker of GFR, May Predict the Early Prognostic Stages of Patients With Type 2 Diabetic Nephropathy

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The relationship between serum levels of β -trace protein (BTP) or serum creatinine (s-Cr) and the prognostic stages of type 2 diabetic nephropathy was determined. 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 (1991, p 251–256) 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. Levels of serum BTP were measured using the nephelometric assay on a BNA II analyzer (Dade Behring Diagnostics, Marburg, Germany). The mean levels of serum BTP in Stage IIIA were significantly higher than those in Stage I or II ($P < 0.00001$, $P < 0.002$, respectively). However, the mean levels of s-Cr in Stage IIIA were not significantly higher than that in Stage I or II. In conclusion, serum BTP was a good marker for the identification of early renal impairment in type 2 diabetes. *J. Clin. Lab. Anal.* 18:237–239, 2004. © 2004 Wiley-Liss, Inc.

Key words: serum β -trace protein; serum creatinine; glomerular filtration rate; type 2 diabetic nephropathy

INTRODUCTION

Glomerular filtration rate (GFR) is the best functional parameter for renal diseases. Inulin and ⁵¹Cr-EDTA plasma clearances are the gold standard methods for the measurement of 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, the levels of Ccr are about 20% lower than those of GFR measured by inulin clearance (C_{In}). s-Cr is influenced by the tubular secretion of creatinine and the patient's muscular mass. Because of such interference, other low molecular weight proteins have been suggested to replace serum creatinine. Serum cystatin C has been advocated as a better predictor of GFR. It is already increased within the so-called creatinine-blind range and independent of age and gender (1). Recently, we reported that the levels of serum cystatin C can predict the early prognostic stage of patients with type 2 diabetic nephropathy (2). Prostaglandin D (PGD) synthase was originally identi-

fied and termed β -trace protein (BTP) by Clausen (3). BTP is a major constituent of human cerebrospinal fluid (CSF), representing approximately 3% of total CSF protein. PGD synthase is an enzyme that catalyzes the conversion of prostaglandin H₂ (PGH₂) to PGD₂. This prostaglandin has various physiologic actions, including sleep induction, nociception, inhibition of platelet aggregation and nitric oxide release, and induction of vasodilation (4,5). BTP belongs to the lipocalin superfamily that is composed of various secretory lipid-transporter proteins. It is a low molecular weight glycoprotein with 168 amino acids and a molecular

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weight of 25 kDa. It is filtered through the glomerular basement membrane, and its concentrations are primarily determined by GFR. BTP has a constant production rate and does not vary in an acute phase reaction. It is considered to be independent of age and gender in the same way as cystatin C.

The purpose of the present study was to determine the relationship between levels of serum BTP and prognostic stages in patients with type 2 diabetic nephropathy.

MATERIALS AND METHODS

Serum samples and classification of type 2 diabetic nephropathy were obtained from 174 patients with type 2 diabetes were obtained from Juntendo University Hospital, Tokyo, and Juntendo Urayasu Hospital, Chiba, Japan. These patients did not show inflammation of the cerebrospinal cord. The serum samples were stored at -20° prior to use. They were classified into four groups according to the Report of the Ministry of Health and Welfare of Japan (6) 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). The patients included 68 in Stage I, 29 in Stage II, 32 in Stage IIIA, 17 in Stage IIIB, and 28 were in Stage IV.

Detection of Serum BTP

The levels of serum BTP were measured using the nephelometric assay on a BNA II analyzer (Dade Behring Diagnostics, Marburg, Germany). This assay is based on the principle of latex particle-enhanced immunonephelometry using rabbit polyclonal antibodies against BTP. Central 95% reference intervals established from a representative cohort of 100 female and 100 male healthy blood donors (median age, 31 years; 2.5–97.5 percentiles, 19.0–60.5 years) were 0.402–0.738 mg/L for BTP (7). The mean levels of BTP in healthy controls were 0.460 ± 0.130 mg/L (mean $\pm$ SD). Levels of s-Cr were measured by routine method (Enzyme assay; CRE-II, Kainos Laboratory Inc., Tokyo, Japan) in our two hospitals. There were no significant changes in the levels of s-Cr between the two hospitals. The mean levels of s-Cr in healthy controls were 0.800 ± 0.200 mg/dl (mean $\pm$ SD).

Statistical Analysis

The mean levels of BTP and creatinine in sera were compared. $P < 0.01$ was regarded as significant.

RESULTS

The mean levels of serum BTP and s-Cr in patients with type 2 diabetes are shown in Tables 1 and 2. The mean levels of serum BTP in Stage I or II were not significantly higher than those in healthy controls. There were no significant changes in the levels of serum BTP between Stage I and II (Fig. 1). The mean levels of

Table 1. Mean levels of serum BTP in patients with type 2 diabetic nephropathy

Stage	Number	Mean $\pm$ SD (mg/dl)
I	68	0.475 ± 0.135
II	29	0.502 ± 0.161
IIIA	32	0.677 ± 0.231
IIIB	17	1.394 ± 0.573
IV	28	4.185 ± 1.79
Healthy controls	200	0.460 ± 0.130

Table 2. Mean levels of serum creatinine in patients 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
Healthy controls	200	0.800 ± 0.200

Fig. 1. Relationship between levels of serum BTP and prognostic stages of type 2 diabetic nephropathy.

Fig. 2. Relationship between levels of serum creatinine (s-Cr) and prognostic stages of type 2 diabetic nephropathy.

serum BTP in Stage IIIA were significantly higher than those in Stage I or II ($P < 0.00001$, $P < 0.002$, respectively). The mean levels of serum BTP in Stage IIIB and Stage IV were also significantly higher than those in Stage I ($P < 0.00001$).

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 among Stages I, II, and IIIA, and in healthy controls (Fig. 2).

DISCUSSION

The most commonly used marker for GFR is serum creatinine. However, s-Cr is an insensitive marker of kidney injury since it is influenced by tubular secretion of creatinine and the patient's muscular mass. C_{In} is the gold standard for evaluating GFR, but it is too expensive and cumbersome for routine clinical use. In recent years, there have been several reports suggesting that low-molecular weight proteins in sera seem to be better markers for detecting reduced GFR than the routine measurement of s-Cr. Several proteins, such as β_2 microglobulin, α_1 microglobulin and cystatin C have been compared with s-Cr (8). Another low-molecular weight protein, BTP, isolated primarily from cerebrospinal fluid, is filtered through the glomerular basement membrane and its concentrations are determined mainly

by GFR (3–5,9). Recently, we reported that an increase of serum cystatin C levels occurred earlier than that of s-Cr in various glomerular diseases (1). We also reported that serum cystatin C was better than s-Cr in terms of sensitivity and specificity as an early prognostic marker of type 2 diabetic nephropathy (2). It appears that the levels of serum cystatin C may provide an early prognostic marker for GFR that is superior to s-Cr.

The results of this study showed a significant relationship between levels of serum BTP and prognostic stages in patients with type 2 diabetic nephropathy. The mean levels of serum BTP in Stage IIIA (macroalbuminuric stage without renal dysfunction) were significantly higher than those in Stage I (normoalbuminuric stage) or II (microalbuminuric stage). The mean levels of s-Cr in Stage IIIA were not significantly higher than those in Stage I or II. These results were similar to those of our previous study using serum cystatin C (2). It is necessary to exclude patients with cerebritis and/or meningitis, or brain tumors, since the levels of serum BTP might be increased by such diseases (3–5,9). It appears that the levels of serum BTP as well as serum cystatin C can predict the early prognostic stage of patients with type 2 diabetic nephropathy.

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