Urinary Excretion of Pancreatic Stone Protein in Diabetic Nephropathy


Urinary pancreatic stone protein (PSP) levels were measured in 68 diabetic patients and 170 healthy controls to investigate the relationship between the progression of diabetic nephropathy and PSP excretion. Urinary albumin, N-acetyl-β-glucosaminidase (NAG), α1-microglobulin, creatinine clearance, and the blood PSP level were also determined in the diabetic patients. The urinary glucose level and glycemic control did not influence the urinary PSP level. In patients with normoalbuminuria (urinary albumin <20 mg/gCr, n=31), microalbuminuria (20–200 mg/gCr, n=19), and macroalbuminuria (>200 mg/gCr, n=18), the mean urinary PSP level was 347, 507, and 860 μg/gCr, respectively. These levels were significantly higher than the level in normal volunteers (168 μg/gCr, p<0.01). A significant positive correlation was observed between the urinary PSP level and the NAG or α1-microglobulin levels (p<0.01). There was a stronger correlation with α1-microglobulin. Blood PSP levels were also elevated in patients who had renal impairment with a decreased creatinine clearance. In conclusion, urinary PSP excretion was increased from the initial stage of diabetic nephropathy and this increase became more marked as nephropathy progressed. Increased PSP excretion may reflect renal tubular dysfunction.

Key words: urinary pancreatic stone protein (PSP), urinary albumin, urinary α1-microglobulin, urinary N-acetyl-β-glucosaminidase (NAG)

Introduction

Pancreatic stone protein (PSP) is a substance which inhibits calcium carbonate precipitation in pancreatic fluid (1, 2). It is a low molecular weight protein (14–19 kd) with five different forms (PSP-S1-5). PSP mRNA is mainly detected in the pancreas, but its expression has also been observed in the gastric mucosa and the kidneys (3). PSP has been found in the urine and renal calculi of healthy individuals (4), suggesting a physiological role of PSP in the kidneys. Previously, we reported that PSP forms, S2-5 are present in the urine of normal individuals and that the PSP levels are correlated with those of N-acetyl-β-glucosaminidase (NAG) (5). We also found that urinary PSP was increased markedly in patients with various renal diseases, including diabetic nephropathy. In some of these patients, urinary PSP was present in forms other than PSP-S2-5 (6). In the present study, we determined urinary PSP levels in patients with diabetes to investigate the mechanism of the increase in urinary PSP excretion, and also assessed the changes of urinary PSP excretion which accompanied the progression of nephropathy.

Materials and Methods

Subjects

The diabetic subjects consisted of 68 outpatients attending the diabetic clinic of Ogaki Municipal Hospital. Their ages ranged between 20 and 84 years (mean: 61 years), and there were 40 men and 28 women. The treatment for diabetes consisted of diet alone in 9 patients, oral hypoglycemic agents in 36 patients, and insulin therapy in 23 patients. The control group consisted of 170 healthy adults with no abnormalities on biochemistry tests and urinalysis who underwent routine annual health checks (60 men and 110 women aged 26–65 years; mean: 45 years).
Urinary PSP in Diabetic Nephropathy

Table 1. Clinical Profiles in Each Group Classified by Urinary Albumin Index

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Age (yr)</th>
<th>Treatment</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM with normoalbuminuria</td>
<td>17/14</td>
<td>58.4 ± 10.7</td>
<td>5/16/10</td>
</tr>
<tr>
<td>DM with microalbuminuria</td>
<td>11/8</td>
<td>65.2 ± 11.5</td>
<td>3/12/4</td>
</tr>
<tr>
<td>DM with macroalbuminuria</td>
<td>12/6</td>
<td>58.1 ± 11.8</td>
<td>1/8/9</td>
</tr>
</tbody>
</table>

OHA: oral hypoglycemic agents.

Methods

Random urine samples were collected to determine the urinary PSP level. In diabetic patients, urinary albumin, NAG, α₁-microglobulin, and urinary glucose were also measured, as were the creatinine clearance (Ccr), the blood hemoglobin A1c level (HbA1c), the blood 1,5-anhydro-D-glucitol (1,5AG) level, and the blood PSP level. Urinary PSP, albumin, NAG, and α₁-microglobulin excretion were all divided by the urinary creatinine level to calculate the respective excretion indices.

Patients were classified into a normoalbuminuria group (urinary albumin index <20 mg/gCr, n=31), a microalbuminuria group (urinary albumin index: 20–200 mg/Ccr, n=19), and a macroalbuminuria group (urinary albumin index >200 mg/gCr, n=18) based on the urinary albumin index data. Clinical profiles in each group are shown in Table 1.

PSP was determined by enzyme immunoassay using a monoclonal antibody (5, 7). Albumin and α₁-microglobulin were determined by radioimmunoassay, NAG, creatinine, and 1,5-AG by colormetry, urinary glucose by electrode assay (electrophoresis), and HbA1c by HPLC.

Results were expressed as the mean (mean±SD). Statistical analysis was conducted by two-way analysis of variance (ANOVA) and Fisher’s multiple comparison method, with p<0.01 being considered to indicate statistical significance.

Results

Urinary PSP in healthy adults and diabetic patients

Since there was a large variation in the urinary PSP levels determined in the healthy controls, log conversion of the data was done and the mean and standard deviation were determined to be 168 (79–411) μg/gCr (8). The mean and standard deviation of the log-converted urinary PSP data were also determined in the diabetic patients. The urinary PSP index for all diabetic patients was 492 (170–1,420) μg/gCr, which was clearly higher than that of the healthy individuals (p<0.01). The correlation coefficients for the relation of urinary PSP with urinary glucose, HbA1c, and 1,5-AG were 0.11, 0.20 and –0.02, respectively, with no significant correlations being observed.

Progression of diabetic nephropathy in relation to urinary albumin and urinary PSP

The urinary PSP index was 347 (104–922), 507 (196–1,325), and 860 (283–2,674) μg/gCr in the normoalbuminuria, microalbuminuria, and macroalbuminuria groups, respectively (Fig. 1). The index was significantly higher than that of the healthy control patients in all groups (*p<0.01). The PSP index of the macroalbuminuria group was also significantly higher than that of the normoalbuminuria group (**p<0.01). C: healthy controls, AU: albuminuria.

Relationship between urinary PSP and urinary NAG, α₁-microglobulin, blood PSP level or Ccr

There was a significant correlation between the urinary PSP and NAG indices in the diabetic patients (r=0.35, p<0.01, n=68) (Fig. 3). The urinary PSP and α₁-microglobulin indices also showed a significant correlation (r=0.68, p<0.01, n=68) (Fig. 4). There was a significant negative correlation between the urinary PSP index and Ccr (r=0.39, p<0.01, n=68) (Fig. 5). There was also a significant negative correlation between the blood PSP concentration and the Ccr (r=–0.52, p<0.01, n=68) (Fig. 6).
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Figure 2. There was a significant correlation between the urinary PSP index and the urinary albumin index.

Figure 4. The urinary PSP and α₁-microglobulin indices also showed a significant correlation.

Figure 3. There was a significant correlation between the urinary PSP and NAG indices in the diabetic patients.

Figure 5. There was a significant negative correlation between the urinary PSP index and Ccr.

Discussion

We have previously reported that urinary PSP excretion is increased in patients with renal disease (6, 8). In the present study, we also confirmed an increase of urinary PSP excretion with the progression of diabetic nephropathy.

Two mechanisms have been suggested to explain the increase of urinary PSP excretion in diabetic nephropathy. PSP is mainly synthesized in the pancreas and is secreted into the pancreatic juice. However, as with various pancreatic enzymes, it can also be found circulating in the blood (7). Since PSP is a low molecular weight protein of 14–19 kd, it crosses the glomerular basement membrane and undergoes reabsorption in the proximal renal tubules, similarly to α₁- and β₂-microglobulin (9, 10). Therefore, increased urinary PSP excretion may indicate abnormal renal tubular function. Another possible mechanism of the increase in urinary PSP excretion is that it may leak into the urine from renal tubular epithelial cells due to some type of cellular injury, as occurs with NAG (11). There are reports that PSP is also synthesized in the kidney and plays some physiological role in this organ. Watanabe et al (3) detected the expression of PSP mRNA in the kidneys, while Verdier et al (4) found that PSP was most abundant in the epithelial cells between the proximal tubule and the ascending branch of the loop of Henle, where it may prevent the precipitation of supersaturated calcium carbonate in the urine (12).

We previously observed that some patients with renal dis-
PSP and urinary glucose, HbA1c, or 1,5-AG it is unlikely that hyperglycemia itself influenced urinary PSP excretion. Therefore, the increase of urinary PSP excretion in the normoalbuminuria group suggests that there is damage to the kidneys, including the renal tubules, even at this early stage. On the other hand, analysis of the macroalbuminuria group showed that there were also cases where PSP excretion did not increase despite severe nephropathy. These results suggest the heterogeneity of diabetic nephropathy. To study the characteristics of the heterogeneity of the nephropathy using various urinary proteins may contribute to the classification of pathogenesis, prognosis and development of prevention in diabetic nephropathy. Further research (including histopathological studies) must be conducted to determine the mechanisms involved.

References