Urinary excretions of lipocalin-type prostaglandin D synthase predict renal injury in type-2 diabetes: a cross-sectional and prospective multicentre study

Yoshio Uehara1, Hirofumi Makino2, Kousuke Seiki3 and Yoshihiro Urade4 on behalf of L-PGDS Clinical Research Group of Kidney

Abstract

Backgrounds. Urinary excretions of lipocalin-type prostaglandin D synthase/β-trace (L-PGDS) probably reflect the increased permeability of injured glomerular capillary walls of the kidney. We tested the hypothesis in cross-sectional and prospective studies that urinary L-PGDS excretions predict renal injury in type-2 diabetes.

Methods. (1) In the cross-sectional studies, we evaluated whether urinary L-PGDS excretions were able to predict renal diseases in a pooled population including 793 healthy subjects and 200 patients with various forms of renal diseases. (2) We determined the cut-off point of urinary L-PGDS excretions to predict ≥30 mg/gCr albuminuria in 666 patients with type-2 diabetes. (3) In the prospective study, 121 type-2 diabetic patients with <30 mg/gCr albuminuria were followed for almost 2 years to examine whether urinary L-PGDS excretions predict the future status of renal injury in type-2 diabetes.

Results. (1) In the cross-sectional studies, receiver operating characteristic analysis revealed that urinary L-PGDS excretions better predicted the patients with kidney diseases than the other markers of renal injury. (2) It was also demonstrated that ≥4.2 mg/gCr urinary L-PGDS excretions better predicted ≥30 mg/gCr albuminuria in type-2 diabetic patients than other markers. (3) The prospective study revealed that in type-2 diabetic patients with <30 mg/gCr albuminuria, the patients with ≥4.2 mg/gCr urinary L-PGDS excretions more likely exhibited the renal injury during the follow-up periods than those with <4.2 mg/gCr urinary L-PGDS excretions.

Conclusions. Urinary L-PGDS excretions reflect the current increased permeability of injured glomerular capillary walls and better predict the future status of renal injury in type-2 diabetes with <30 mg/gCr albuminuria.

Keywords: albuminuria; biomarker; diabetes mellitus; nephropathy; prostaglandin D synthase

Introduction

Diabetic nephropathy has been one of the leading causes of end-stage renal disease (ESRD) over the past 10 years in Japan. Much evidence suggests that albuminuria per se worsens the prognosis of cardiovascular events in various systemic diseases. If we successfully recognize the risk of albuminuria or proteinuria in advance, and if we treat such patients more meticulously, it might be useful to improve the prognosis in such patients. In fact, it is acknowledged that ≥30 mg/gCr albuminuria suggests current renal injury in diabetes [1–6]. Unfortunately, however, in the patients with <30 mg/gCr albuminuria, there have been no useful markers indicating that the renal injury occurs or is impending. The assessment of the status of renal injury in such an albuminuria-blind range would be helpful to improve the prognosis of diabetic nephropathy through stricter management.

Lipocalin-type prostaglandin D synthase/β-trace (L-PGDS) is an enzyme-synthesizing prostaglandin D2 (PGD2) and a secretory protein of the lipocalin superfamily [7,8]. It is synthesized in choroid plexus or leptomeninges in the brain and secreted steadily through cerebrospinal fluid into circulating blood. L-PGDS is similar to serum albumin in the chemical properties including anionic charges at pH 7.4; however, the molecular weight is much smaller than serum albumin (26 000 versus 66 000 Da). Thus, L-PGDS more easily passes through glomerular capillary walls of the kidney than serum albumin. In fact, we have previously demonstrated that the glomerular clearance rate of L-PGDS is ~6% of the creatinine clearance rate in
normal rats while the clearance of albumin is negligible [9]. Considering these data, it seems probable that urinary L-PGDS excretions reflect a slight change in the permeability of glomerular capillary walls.

Since serum L-PGDS is excreted through glomerular capillary walls, reduction of the number of functioning glomeruli decreases the renal clearance of L-PGDS and increases serum L-PGDS concentrations [10,11]. Considering these data, urinary L-PGDS excretions might be more useful to predict the increased glomerular permeability in an early stage of systemic diseases rather than in advanced kidney injury. In this context, we have demonstrated that urinary L-PGDS excretions increase in a microalbuminuric stage of type-2 diabetes, and the excretions are reversed towards normal levels with a strict control of blood sugar levels [12,13]. Moreover, Hirawa et al. have demonstrated that hypertensive patients have greater urinary L-PGDS excretions than normotensives even when they are apparently free from overt proteinuria [14].

Based on a series of previous studies, in the present study, we proposed the hypothesis that urinary L-PGDS excretions are a better marker of the early glomerular damage in type-2 diabetes. To test this hypothesis, we examined in two cross-sectional studies whether urinary L-PGDS excretions were useful or not to predict primary renal diseases, and furthermore, to predict ≥30 mg/gCr albuminuria in type-2 diabetes. In addition, in the prospective study, for almost 2 years we investigated the predictability of urinary L-PGDS excretions for the future status of the renal injury in type-2 diabetic patients with <30 mg/gCr albuminuria.

Subject and methods

Protocol of the studies

Cross-sectional studies on L-PGDS in urine. To disclose the basal profile of urinary L-PGDS excretions, in the cross-sectional studies, we measured urinary L-PGDS excretions in healthy subjects who visited respective clinics for medical checkup and patients under medical management for renal diseases or type-2 diabetes. The patients had been treated with medications at responsible doctors’ discretion. They visited each clinic at regular intervals. Medical records were referred to obtain information on their basal diseases. Blood and spot urine samples were collected at their regular visits as described below.

L-PGDS in normal subjects

Three hundred and forty-three male subjects aged 20–76 with median 49, and 450 female subjects aged 22–72 with median 44, were enrolled as the normal group. These subjects visited the hospitals for annual medical checkup, and eventually they were found free from apparent diseases including renal or systemic cardiovascular diseases or hormonal or metabolic disorders.

L-PGDS in patients with renal diseases

We examined whether urinary L-PGDS excretions predicted patients with renal diseases, and furthermore, attempted to determine the cut-off point with the highest accuracy to predict renal diseases. One hundred and nine male and 91 female patients were definitely diagnosed as having renal diseases by blood biochemical or immunological studies, urinalyses, CT and MRI, or if necessary, by a renal biopsy. They had been treated at doctors’ discretion in each outpatient hospital on a monthly basis. The spot urine and blood samples were obtained at their regular visits.

The usefulness of urinary L-PGDS excretions was assessed using receiver operating characteristic (ROC) analysis.

L-PGDS and type-2 diabetes

We examined whether urinary L-PGDS excretions predicted ≥30 mg/gCr albuminuria in type-2 diabetic patients. Six hundred and sixty-six patients diagnosed as having type-2 diabetes were enrolled in the diabetic arm of the cross-sectional studies. These patients had been treated at doctors’ discretion in each outpatient hospital. The spot urine and blood samples were collected at their regular visits. The classification value for significant renal injury was set at ≥30 mg/gCr albuminuria according to the criteria of the American Diabetes Association [15]. The usefulness of urinary L-PGDS excretions was assessed using ROC analysis.

Prospective study on predictability of L-PGDS for diabetic microalbuminuria. One hundred and thirty type-2 diabetic patients with <30 mg/gCr albuminuria were enrolled in the prospective study, and finally, 121 cases finished the 2-year prospective study with complete data from renal and metabolic examinations. After the recruitment, doctors were not allowed to give any new forms of RAS inhibitors including ACEI and ARB, or to change the doses that had been given throughout the prospective study. Moreover, the doctors involved in the study and the patients were blind to the measurements of urinary L-PGDS. The group assigned based on urinary L-PGDS was not disclosed to the responsible doctors or the patients until the study was finished.

Using the cut-off point of urinary excretions of L-PGDS with the highest accuracy to predict albuminuria, which was determined in the diabetic arm of the cross-sectional studies, we examined how well urinary L-PGDS excretions predicted future renal injury.

Responsible doctors continued to manage diabetes at their discretion on a monthly basis throughout the study. At the end of the prospective study, spot urine and blood specimens were obtained for the examination. The usefulness of urinary L-PGDS excretions was assessed using ROC analysis.

L-PGDS determination by the latex method

Urinary L-PGDS was determined according to the previous methods [12–14]. Briefly, reagent 1 consisted of a 0.2 mol/L PIPES buffer (pH 7.0) containing 0.1% BSA and 1% NaCl. Reagent 2 consisted of a 50 mmol/L borate buffer (pH 7.3) containing 0.2% latex sensitized with an anti-human L-PGDS rabbit polyclonal antibody. Six microlitres of sample was mixed with 60 µL of 0.2 mol/L PIPES buffer (pH 7.0) containing 0.1% BSA and 1%
NaCl and incubated at 37°C for 5 min. Sixty microlitres of 50 mmol/L borate buffer (pH 7.3) containing 0.2% latex sensitized with an anti-human L-PGDS rabbit polyclonal antibody was added. After incubation at 37°C for 5 min, the density was measured at a wavelength of 571 nm by an autoanalyser (JCA BM-1250, JEOL Ltd, Tokyo, Japan). Urinary L-PGDS excretions were expressed as milligrams per gram urinary creatinine excretions (mg/gCr). In our preliminary study, we have found that urinary L-PGDS excretions in spot urine expressed as mg/gCr well correspond with the measurements in 24-h collected urine [13]. The intra-assay and inter-assay coefficients of variances were 0–0.9% and 0.4–2.8%, respectively.

Markers of renal damage

Urinary type-IV collagen excretions were measured by an enzyme immunoassay (Panauia uIV kit, Fuji Chemical Industries, Ltd, Toyama, Japan) according to the manufacturer’s instructions [16]. The measurements were expressed as µg/gCr. The other biochemical markers were measured with an autoanalyser (Model Hitachi 736, Hitachi Co., Ltd, Tokyo, Japan). HbA1c was determined by high-performance liquid chromatography. Albumin in urine was measured by the standard immunoprecipitation method [12–14].

Statement of clinical studies

This study was in accordance with the guidelines for human studies in the respective hospitals and clinics, and approved by the respective authoritative boards of hospitals and departments. Informed consent was obtained in a written form from all subjects upon entry.

Statistical analysis

The values were expressed as a distribution range with median or means ± standard deviation (SD). Homology of variance was assessed by the Brown–Forsythe test. To assess differences, we utilized non-parametric tests including the Mann–Whitney U-test, Kruskal–Wallis ANOVA by Ranks and Spearman-rank order correlations using the software STATISTICA version 6 (StatSoft, Tulsa, OK, USA). The ROC curve was analysed using MedCalc (MedCalc Software, Mariakerke, Belgium) [17]. P-values of <0.05 were considered as statistically significant.

Results

The cross-sectional studies

Urinary L-PGDS excretions in normal subjects. Distribution of urinary L-PGDS excretions in the male (n = 343) and the female healthy subjects (n = 450) are shown in Figure 1. Urinary L-PGDS excretions ranged from 0.1 to 10.9 (median 1.7) mg/gCr in the male and from 0.1 to 22.3 (median 1.1) mg/gCr in the female subjects (Mann–Whitney U-test, P < 0.001). Urinary L-PGDS excretions were weakly correlated with age in the female subjects (Spearman rank order correlations were 0.212 in the female, P < 0.05, and 0.038 in the male subjects, P > 0.1).

Urinary L-PGDS excretions in patients with renal diseases. We examined urinary L-PGDS excretions in 200 patients (male, 91; female, 109) diagnosed as having renal diseases. We recruited the patients with proven renal diseases and the normal subjects from the several affiliated clinics. The doctors participating in this study were in charge of the department of nephrology or haemodialysis units or diabetic care divisions. Such specialty might increase the prevalence of renal diseases in our hospitals, which is much greater than the general prevalence of 0.5% based on data from medical checkups in Japan.

The patients with renal diseases, on the whole, had greater urinary L-PGDS excretions than the normal subjects (Table 1). Moreover, the patients with any form of renal diseases, except for IgA nephritis in the male patients, exhibited higher urinary L-PGDS excretions than the healthy subjects.

We assessed the predictability of renal diseases using a combined population of the healthy subjects and the patients with renal diseases. An increase of the cut-off point of urinary L-PGDS excretions decreased its sensitivity and increased the specificity. The cut-off point with the highest accuracy and with minimal false negative and false positive errors was 2.9 mg/gCr [sensitivity, 67.0% with 56.4–76.5% (95% CI); specificity, 93.3% with 90.6–95.5% (95% CI)] in the female and 3.2 mg/gCr [sensitivity, 67.0% with 57.3–75.7% (95% CI); specificity, 86.0% with 81.8–89.5% (95% CI)] in the male patients.

We compared the accuracy of urinary L-PGDS excretions with other markers of renal injury (Figure 2). The results from the pairwise comparison of ROC curves are summarized in Table 2. The ROC area of urinary L-PGDS excretions was second to albuminuria, and greater than...
Table 1. Urinary excretions of L-PGDS in normal subjects and patients with renal diseases

<table>
<thead>
<tr>
<th>Groups</th>
<th>Female (mg/gCr)</th>
<th>Male (mg/gCr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median Range</td>
<td>Median Range</td>
</tr>
<tr>
<td>Normal</td>
<td>1.1 0.1–6.1</td>
<td>1.7 0.1–10.9</td>
</tr>
<tr>
<td>Renal</td>
<td>3.7 0.4–41.4</td>
<td>5.0 0.2–44.6</td>
</tr>
<tr>
<td>CGN</td>
<td>3.6 1.1–13.1</td>
<td>4.7 0.6–13.2</td>
</tr>
<tr>
<td>IgA</td>
<td>2.4 0.4–14.7</td>
<td>1.9 0.2–29.0</td>
</tr>
<tr>
<td>MN</td>
<td>8.5 3.5–14.2</td>
<td>4.6 1.1–11.2</td>
</tr>
<tr>
<td>SK</td>
<td>–</td>
<td>6.4 1.3–14.2</td>
</tr>
<tr>
<td>PK</td>
<td>3.7 3.2–14.3</td>
<td>5.5 3.5–17.7</td>
</tr>
<tr>
<td>CRF</td>
<td>11.6 1.8–41.4</td>
<td>16.0 0.9–44.6</td>
</tr>
<tr>
<td>IN</td>
<td>4.2 0.7–18.4</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Cases</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>450</td>
<td>343</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>91</td>
<td>109</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CGN</td>
<td>26</td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgA</td>
<td>32</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>MN</td>
<td>3</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>SK</td>
<td>0</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>PK</td>
<td>4</td>
<td>5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CRF</td>
<td>15</td>
<td>31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IN</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

CGN, chronic glomerulonephritis (pathological diagnosis unknown); IgA, IgA nephritis; MN, minimal change glomerulonephritis; SK, sclerosing nephritis; PK, polycystic kidney; CRF, chronic renal failure; IN, interstitial nephritis; NS, not significant.
The range indicates minimal and maximal measurements of urinary excretions of L-PGDS.

The differences were assessed by the Kolmogorov–Smirnov test or the Kruskal–Wallis analysis of ranks and the median test.

Table 2. Comparison of area of variables on ROC analysis to predict renal diseases in normal subjects and patients with renal diseases in the cross-sectional study

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUC</th>
<th>SE</th>
<th>95% CI</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPGDS</td>
<td>0.840</td>
<td>0.019</td>
<td>0.809–0.868</td>
<td></td>
</tr>
<tr>
<td>uAlb</td>
<td>0.961</td>
<td>0.010</td>
<td>0.943–0.975</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uIVC</td>
<td>0.745</td>
<td>0.022</td>
<td>0.710–0.779</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ub2M</td>
<td>0.730</td>
<td>0.023</td>
<td>0.694–0.764</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uNAG</td>
<td>0.661</td>
<td>0.024</td>
<td>0.623–0.698</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sCr</td>
<td>0.518</td>
<td>0.020</td>
<td>0.478–0.548</td>
<td>NS</td>
</tr>
</tbody>
</table>

AUC, area under the ROC curve; SE, standard error; 95% CI, 95% confidence intervals; uPGDS, urinary excretions of L-PGDS; uAlb, urinary excretions of albumin; uIVC, urinary excretions of type-IV collagen; ub2M, urinary excretions of beta-2 microglobulin; uNAG, urinary excretions of NAG; sCr, serum levels of creatinine.

Table 2. Comparison of area of variables on ROC analysis to predict renal diseases in normal subjects and patients with renal diseases in the cross-sectional study

Fig. 2. ROC analysis of markers to predict renal diseases. This illustration demonstrated the accuracy of urinary L-PGDS excretions as a marker of renal diseases.

Urinary L-PGDS excretions in type-2 diabetes. Accordingly, we examined whether urinary L-PGDS excretions accurately predicted ≥30 mg/gCr albuminuria in type-2 diabetic patients. Six hundred and sixty-six patients (281 female and 385 male), diagnosed as having type-2 diabetes, were investigated. Two hundred and thirty-one patients had ≥30 mg/gCr albuminuria and 435 patients had <30 mg/gCr. These patients had been treated at doctors’ discretion in each affiliated outpatient hospital.

An increase in the cut-off point of urinary L-PGDS excretions decreased the sensitivity to predict ≥30 mg/gCr albuminuria, and increased the specificity. The cut-off point with the highest accuracy was 2.8 mg/gCr [sensitivity, 83.8% with 75.3–90.3% (95% CI); specificity, 71.6% with 64.3–78.1% (95% CI)] in the female and 4.2 mg/gCr [sensitivity, 56.3% with 47.2–65.2% (95% CI); specificity, 75.7% with 70.0–80.8% (95% CI)] in the male patients.

When the cut-off point was set at 4.2 mg/gCr in the female patients, the sensitivity decreased from 83.8% to 59.0% with 49.0–68.5% (95% CI) and the specificity increased from 71.6% to 88.1% with 82.3–92.5% (95% CI). Thus, at the cut-off point of 4.2 mg/gCr, the false negative was 41% for the female and 44% for the male patients, and the false positive was 12% for the female and 24% for the male patients.

Moreover, we compared the accuracy with other markers of renal damage using ROC analysis and the pairwise comparison (Figure 3 and Table 3). Urinary L-PGDS excretions were superior to the other markers tested in order to predict ≥30 mg/gCr albuminuria in type-2 diabetic patients.

The prospective study on urinary L-PGDS excretions

The cross-sectional studies suggested that the cut-off point of 4.2 mg/gCr better predicted ≥30 mg/gCr albuminuria in type-2 diabetic patients. At this cut-off point, ∼12% of...
the end,

versus 138

and the high-L-PGDS groups (6.9

of the low- and high-L-PGDS groups were 135

at the entry,

for the high-L-PGDS group (median 603 days (25–75% quantile, 586–750 days) for the low-L-PGDS group, the patients with

microglobulin excretions remained higher after 12 months in the high-L-PGDS group. At the end of the study, urinary beta-2 microglobulin

excretions and serum creatinine levels were greater in the high-L-PGDS group than in the low-L-PGDS group. Thus,

excretions possibly indicate an occurrence of glomerular injury in type-2 diabetic patients.

This discrepancy might be due to the real type-2 error or to

false positive. In other words, urinary L-PGDS excretions

were 80 ± 12 versus 78 ± 10 mmHg at the entry,

P = 0.574, and 77 ± 11 versus 76 ± 11 mmHg, P = 0.786, respectively.

At the entry, the markers including albuminuria, urinary type-IV collagen excretions, and urinary beta-2 microglobulin

excretions were significantly higher in the high-L-PGDS group than the low-L-PGDS group (Table 4). The high-L-PGDS group had higher albuminuria than the low-L-PGDS group upon entry and at the end, although the differences were very small. The distribution of albuminuria at the end of the study was median 9.8 mg/gCr (min/max, 1.6–116.0 mg/gCr and 90 percentile, 25.1 mg/gCr) for the low-PGDS group and median 14.1 mg/gCr (min/max, 0.2–162.0 and 90 percentile 58.8 mg/gCr) for the high-PGDS group. A number of the cases were well within 30 mg/gCr, and much less were ≥ 30 mg/gCr. The albuminuria was not distributed normally either in the low- or high-PGDS group.

Such particular distributions were responsible for the slight difference in albuminuria between the two groups. More interestingly, the high-L-PGDS group more likely exhibited ≥30 mg/gCr albuminuria than the low-PGDS group during the follow-up periods, and finally, at the end of the study, the difference was statistically significant. Urinary type-IV collagen and urinary beta-2 microglobulin excretions remained higher after 12 months in the high-L-PGDS group. At the end of the study, urinary beta-2 microglobulin excretions and serum creatinine levels were greater in the high-L-PGDS group than in the low-L-PGDS group. Thus, the cut-off point of 4.2 mg/gCr urinary L-PGDS excretions was helpful to predict the current and future renal injury in type-2 diabetes with <30 mg/gCr albuminuria.

Since urinary L-PGDS excretions better predicted renal injury in type-2 diabetes, we attempted to reveal using ROC analysis what markers are predictable of 4.2 mg/gCr urinary L-PGDS excretions at the end of the study. As shown in Table 5, urinary L-PGDS excretions at the entry were superior to the other makers.

Discussion

Microalbuminuria (30–300 mg/gCr) is a significant risk factor for cardiovascular events and a useful predictor of the concomitant renal injury in diabetic patients [15,18,19]. Albuminuria reflects the increased permeability of glomerular capillary walls in various systemic disorders. Unfortunately, however, markers have not been available to assess the renal injury in diabetes in an albuminuria-blind range (<30 mg/gCr albuminuria). If an early stage of the renal injury were recognized, meticulous management of diabetes would possibly prevent the onset of overt albuminuria and progression to ESRD. Thus, it seems quite important clinically to explore sensitive markers for an early change in the kidney function in diabetic patients.

We have previously reported that urinary L-PGDS excretions possibly indicate an occurrence of glomerular injury in type-2 diabetic patients [12,13]. Urinary L-PGDS is believed to reflect a slight change in glomerular permeability because of the smaller molecular weight and its anionic property. We have demonstrated using a genetic rat model of type-2 diabetes, Otsuka Long Evans Tokushima (OLETF) rat, that the glomerular filtration rate of L-PGDS

Fig. 3. ROC analysis of markers to predict albuminuria in type-2 diabetes. This illustration demonstrated the accuracy of urinary L-PGDS excretions to predict ≥30 mg/gCr albuminuria in type-2 diabetic patients.

Table 3. Comparison of area of variables on ROC analysis to predict albuminuria in type-2 diabetes in the cross-sectional study

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUC</th>
<th>SE</th>
<th>95% CI</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPGDS</td>
<td>0.759</td>
<td>0.021</td>
<td>0.725–0.791</td>
<td>–</td>
</tr>
<tr>
<td>uIVc</td>
<td>0.723</td>
<td>0.022</td>
<td>0.687–0.756</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>ub2M</td>
<td>0.696</td>
<td>0.022</td>
<td>0.660–0.731</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>uNAG</td>
<td>0.670</td>
<td>0.023</td>
<td>0.632–0.705</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>sCr</td>
<td>0.540</td>
<td>0.024</td>
<td>0.502–0.579</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

AUC, area under the ROC curve; SE, standard error; 95% CI, 95% confidence intervals; uPGDS, urinary excretions of L-PGDS; uIVc, urinary excretions of type-IV collagen; ub2M, urinary excretions of beta-2 microglobulin; uNAG, urinary excretions of NAG; sCr, serum levels of creatinine.

*P versus area of urinary excretions of PGDS in a pairwise comparison of ROC curves.

the female and 24% of the male patients were classified as false positive. In other words, urinary L-PGDS excretions were ≥4.2 mg/gCr while albuminuria was <30 mg/gCr. This discrepancy might be due to the real type-2 error or to masked renal injury in an albuminuria-blind range.

To make this point clear, the patients with <30 mg/gCr albuminuria were classified into two groups, (1) low-L-PGDS group, the patients with <4.2 mg/gCr urinary L-PGDS excretions and (2) high-L-PGDS group, the patients with ≥4.2 mg/gCr. The patients were followed up for median 715 days (25–75% quantile, 586–750 days) for the low-L-PGDS group and median 603 days (25–75% quantile, 575–719 days) for the high-L-PGDS group (P > 0.1).

There were no differences in HbAlc between the low- and the high-L-PGDS groups (6.9 ± 1.3 versus 7.1 ± 1.1% at the entry, P = 0.386, and 7.5 ± 2.1 versus 7.2 ± 1.1 at the end, P = 0.348, respectively). Systolic blood pressures of the low- and high-L-PGDS groups were 135 ± 17 versus 138 ± 16 mmHg at the entry, P = 0.575, and 135 ± 14 versus 138 ± 19 mmHg at the end, P = 0.574. Diastolic pressures were 80 ± 12 versus 78 ± 10 mmHg at the entry,
Table 4. Changes in the markers of renal injury during the study

<table>
<thead>
<tr>
<th>Variables (units)</th>
<th>Periods</th>
<th>&lt;4.2 mg/gCr (77)</th>
<th>≥4.2 mg/gCr (44)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPGDS (mg/gCr)</td>
<td>Entry</td>
<td>2.45 (0.2–4.2)</td>
<td>5.5 (4.2–10.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>2.6 (0.0–8.8)</td>
<td>4.9 (0.6–13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>2.8 (0.3–8.3)</td>
<td>4.6 (1.2–11.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uAlb (mg/gCr)</td>
<td>Entry</td>
<td>8.1 (0.4–25.2)</td>
<td>14.8 (0.8–29.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>8.9 (0.4–179.0)</td>
<td>12.9 (0.3–63.9)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>9.8 (1.6–116.0)</td>
<td>14.1 (0.2–162.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>uAlb ≥30 mg/gCr (%)</td>
<td>Entry</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>6.5</td>
<td>15.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>6.5</td>
<td>25.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>uIVc (µg/gCr)</td>
<td>Entry</td>
<td>4.3 (0.9–12.3)</td>
<td>6.4 (1.0–19.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>4.0 (0.7–24.0)</td>
<td>6.4 (1.0–15.8)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>3.8 (0.7–13.9)</td>
<td>6.1 (0.4–34.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ub2M (g/L)</td>
<td>Entry</td>
<td>86 ± 86</td>
<td>172 ± 146</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>128 ± 240</td>
<td>230 ± 319</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>138 ± 185</td>
<td>223 ± 299</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>uNAG (U/L)</td>
<td>Entry</td>
<td>4.74 ± 5.22</td>
<td>3.78 ± 3.00</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>5.18 ± 5.25</td>
<td>4.50 ± 3.48</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>6.81 ± 17.3</td>
<td>5.33 ± 3.71</td>
<td>NS</td>
</tr>
<tr>
<td>sCr (mg/dL)</td>
<td>Entry</td>
<td>0.74 ± 0.19</td>
<td>0.80 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>0.73 ± 0.19</td>
<td>0.79 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>0.73 ± 0.23</td>
<td>0.84 ± 0.31</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

uPGDS, urinary excretions of L-PGDS; uIVc, urinary excretions of type-4 collagen; ub2M, urinary excretions of beta-2 microglobulin; uNAG, urinary excretions of NAG; sCr, serum levels of creatinine.

Values were expressed as median with a range of minimum to maximum or means ± SD. Homogeneity of variance was assessed by the Brown–Forsythe test.

The differences were assessed by parametric ANOVA or non-parametric Kruskal–Wallis ANOVA by ranks.

Table 5. Comparison of the ROC area to predict urinary L-PGDS excretions at the end of the study

<table>
<thead>
<tr>
<th>Variables at the entry</th>
<th>AUC</th>
<th>SE</th>
<th>95% CI</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPGDS</td>
<td>0.983</td>
<td>0.014</td>
<td>0.941–0.997</td>
<td>–</td>
</tr>
<tr>
<td>uAlb</td>
<td>0.720</td>
<td>0.050</td>
<td>0.631–0.798</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uIVc</td>
<td>0.736</td>
<td>0.049</td>
<td>0.648–0.812</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ub2M</td>
<td>0.735</td>
<td>0.049</td>
<td>0.647–0.811</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>uNAG</td>
<td>0.543</td>
<td>0.055</td>
<td>0.450–0.633</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sCr</td>
<td>0.530</td>
<td>0.055</td>
<td>0.437–0.621</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AUC, area under the ROC curve; SE, standard error; 95% CI, 95% confidence intervals; uPGDS, urinary excretions of L-PGDS; uAlb, urinary albumin excretions; uIVc, urinary excretions of type-4 collagen; ub2M, urinary excretions of beta-2 microglobulin; uNAG, urinary excretions of NAG; sCr, serum levels of creatinine.

The classification at the end point was 4.2 mg/gCr or more excretions of L-PGDS in urine according to the cut-off point suggested in the cross-sectional study of type-2 diabetes stated in the text.

*P versus area of urinary excretions of PGDS in pairwise comparison of ROC curves.

is ~6% of the creatinine clearance rate (Ccr) in the non-diabetic, control Long Evans Tokushima Otsuka (LETO) rat. The filtration rate of L-PGDS increases up to 14% in the established stage of type-2 diabetes [9].

We demonstrated that urinary L-PGDS excretions increased in the patients with various forms of renal diseases, as compared with the normal subjects. The ROC analysis revealed that in order to predict the patients with renal diseases, urinary L-PGDS excretions were superior to the other markers including urinary excretions of type-IV collagen, beta-2 microglobulin and NAG and serum creatinine concentrations. The urinary L-PGDS excretions were positively related to albuminuria in such patients (r = 0.36, P < 0.05). Moreover, multiple regression analysis revealed that urinary L-PGDS excretions were independently determined by urinary type-IV collagen excretions (β = 0.38, P < 0.0001), serum creatinine levels (β = 0.42, P < 0.0001), albuminuria (β = 0.13, P < 0.001) and urinary beta-2 microglobulin excretions (β = 0.16, P < 0.01), thereby suggesting that the increase in urinary L-PGDS excretions was related to the pathophysiological process responsible for the injury in the glomerular capillary walls [9–11].

Since urinary L-PGDS excretions probably better reflected the changes in glomerular capillary walls in primary renal diseases, we extended our study to investigate the usefulness to predict the renal injury in type-2 diabetes. We demonstrated that urinary L-PGDS excretions better predicted ≥30 mg/gCr albuminuria in type-2 diabetes. Moreover, the ROC analysis revealed that urinary L-PGDS excretions were superior to the other markers including urinary excretions of type-IV collagen, beta-2 microglobulin and NAG and serum creatinine levels. The cut-off point set at 4.2 mg/gCr was the best for both male and female diabetic patients.

In the cross-sectional study on diabetes, 12% of the female patients, and 24% of the males, had ≥4.2 mg/gCr urinary L-PGDS excretions while albuminuria was well within 30 mg/gCr. This might be due to either the real type-2 error or the masked injury in an albuminuria-blind range. To address this point clearly, we followed up the two subgroups of the type-2 diabetic patients with <30 mg/gCr albuminuria.
Appendix

L-PGDS Clinical Research Group of Kidney

Yasu Totsuka
Atsushi Numabe
Kazuhiro Uijke
Yasuhi Yamasaki
Toshi Ieda
Tomoko Gomi
Miho Ajima
Kenji Takahashi
Takashi Matsumuka
Kosuke Seki, Hiroshi Nakajima, Hiroshi Oda,
Yasuhiro Shina, Takamasa Tsuchida
Minoru Yamakado
Kosuke Ohta
Kazuhi Tanai, Saeki Ogawa
Jun Wada, Kenichi Shikata, Hirofumi Makino
Yoshihiro Urade, Naomi Eguchi
Yutaka Eguchi
Kumiko Hamano
Eiko Takahashi
Masao Takagi
Yoshiio Uehara, Rie Hakamada-Taguchi,
Hideyuki Negoro, Yukari Kawabata, Yaqiong Wu
Yumiko Ikeda
Nobuhiro Hirawa, Yoshiyuki Toya, Nobuyoshi Takagi,
Minoru Kihara, Satoshi Umemura
Mikio Sato
Toshinari Ohashi, Ippei Nishimura

Chofu Touzan Hospital, Tokyo, Japan
Department of Clinical Laboratory Medicine, Dokkyo
Medical University School of Medicine, Mibu, Japan
Department of Medicine, Himeji Red Cross Hospital, Himeji, Japan
Department of Medicine, Hiroshima City Hospital. Hiroshima, Japan
Jingumae Clinic, Tokyo, Japan
Department of Endocrinology and Metabolism and Department of
Nephrology, Kanto Medical Center, Tokyo, Japan
Kawasaki Saiwai Clinic, Kawasaki, Japan
Department of Diabetes, Kurashiki Central Hospital, Kurashiki, Japan
Kurashiki Life Style Diseases Center, Kurashiki-Heisei Hospital, Kurashiki, Japan
Central Research Institute, Maruha Group, Tsukuba, Japan
Health Service Center Mitsui Hospital, Tokyo, Japan
Department of Medicine, National Okayama Medical Center, Okayama, Japan
Department of Medicine, Okayama Central Hospital, Okayama, Japan
Department of Medicine and Clinical Science, Okayama University Graduate
School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
Department of Molecular Behavioral Biology, Osaka Bioscience Institute,
Osaka, Japan
Department of Clinical and Intensive Care Medicine, Shiga University of
Medical Science, Shiga, Japan
Shonan Kamakura General Hospital, Kamakura, Japan
Department of Hygiene and Preventive Medicine, Showa University School of
Medicine, Tokyo, Japan
Department of Medicine, Tokyo Metropolitan Police Hospital, Tokyo, Japan
Health Service Center Univ. of Tokyo, Tokyo, Japan
Department of Medicine #2, Yokohama Minami Kyoai Hospital, Yokohama,
Japan
Department of Medical Science and Cardiorenal Medicine, Yokohama
City University Graduate School of Medicine, Yokohama, Japan
Department of Medicine, Yura Hospital, Okayama, Japan
Diagnostics Research Laboratories, Wako Pure Chemical Industries, Ltd,
Amagasaki, Japan

albuminuria, i.e. the low-L-PGDS (<4.2 mg/gCr urinary L-PGDS excretions) and the high-L-PGDS (≥4.2 mg/gCr) groups. The patients with ≥4.2 mg/gCr urinary L-PGDS excretions more likely exhibited higher levels of the markers of renal injury than those with <4.2 mg/gCr urinary L-PGDS excretions. More interestingly, the patients with ≥30 mg/gCr albuminuria increased in number in the high-L-PGDS group than in the low-L-PGDS group during the study. In ~2 years, one-fourth of the patients with ≥4.2 mg/gCr urinary L-PGDS excretions developed ≥30 mg/gCr albuminuria while in the case of <4.2 mg/gCr urinary L-PGDS excretions, only 5% exhibited ≥30 mg/gCr albuminuria. Since the blood sugar levels and blood pressures were well controlled in both groups, the differences in the markers were not due to the differences in the management of diabetes. The cut-off point of 4.2 mg/gCr urinary L-PGDS excretions was clinically useful to predict the current and future renal injury in type-2 diabetic patients. Moreover, the ROC analysis also demonstrated that in order to predict future 4.2 mg/gCr urinary L-PGDS excretions, urinary L-PGDS excretions were the most accurate marker of diabetes.

Finally, the cut-off point of 4.2 mg/gCr urinary L-PGDS excretions is more useful to find early renal injury in an albuminuria-blind range. In the combination of ≥30 mg/gCr albuminuria, urinary L-PGDS excretions increase the power to detect the early stage of renal injury in type-2 diabetes probably through sensing of the increased permeability of damage glomerular capillary walls.

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Conflict of interest statement. None declared.

References


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