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Increased Urinary Angiotensinogen Is Precedent to Increased Urinary Albumin in Patients With Type 1 Diabetes

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Abstract

Background—We previously reported that kidney and urinary angiotensinogen levels were significantly increased before the development of diabetic nephropathy in diabetic rats. To address this system in humans, we have developed an enzyme-linked immunosorbent assay for human angiotensinogen and reported that urinary excretion of angiotensinogen levels is enhanced in patients with chronic kidney disease, including patients with type 2 diabetes. On the basis of these findings, this study was performed to demonstrate that urinary angiotensinogen levels increased before the onset of microalbuminuria and that urinary angiotensinogen can be an early biomarker of intra-renal renin-angiotensin system status in normoalbuminuric patients with type 1 diabetes compared with age- and sex-matched control subjects.

Methods—The study included 28 patients with type 1 diabetes and 21 control subjects. No subject received renin-angiotensin system blockades. Random spot urine samples as well as blood samples were obtained and analyzed.

Results—Urinary albumin:creatinine ratio or urinary protein:creatinine ratio did not increase in patients compared with control subjects, suggesting that these patients were in their premicroalbuminuric phase of diabetic nephropathy. However, the urinary angiotensinogen:creatinine ratio was significantly higher in patients than in control subjects (12.1 ± 3.2 μg/g versus 4.2 ± 0.7 μg/g). Importantly, an increase in plasma angiotensinogen levels was not observed (26.3 ± 1.3 μg/mL versus 29.5 ± 3.3 μg/mL).

Conclusions—Thus, in patients, an increase in urinary angiotensinogen levels is observed, and this increase is precedent to an increase in urinary albumin levels, suggesting that urinary angiotensinogen may function as an early marker of diabetic nephropathy.

Key Indexing Terms

Angiotensinogen; Enzyme-linked immunosorbent assay; Renin-angiotensin system; Type 1 diabetes; Clinical study

Diabetic nephropathy is the most common cause of end-stage renal failure in patients undergoing dialysis in developed countries.[1] Until recently, diabetic nephropathy was thought to be a unidirectional process that starts with microalbuminuria and leads to end-stage renal

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failure.[2] However, it was recently shown that a large proportion of patients diagnosed with diabetic nephropathy reverted to normoalbuminuria, one third of whom lost renal function even during the microalbuminuric stage.[3] Therefore, a more sensitive and specific marker for diabetic nephropathy is needed.

The focus of interest on the renin-angiotensin system (RAS) has shifted to the investigation of its role in specific tissues.[4] The mechanism of the tissue RAS in the kidney is unique because the components necessary to generate angiotensin II are produced within the kidney.[5] It has been postulated that the intrarenal RAS plays an important role in the pathogenesis of diabetic nephropathy.[6,7] Although most of the circulating angiotensinogen is produced and secreted by the liver, the kidneys also produce angiotensinogen.[8] In diabetic animal models, kidney angiotensinogen levels have been shown to significantly increase in streptozotocin-induced type 1 diabetic rats[9] as well as in Zucker diabetic fatty obese rats.[10] Moreover, we previously reported that kidney and urinary angiotensinogen levels were significantly higher in diabetic rats before the development of diabetic nephropathy.[11] To investigate this relationship in humans, we have previously developed an enzyme-linked immunosorbent assay (ELISA) specific for evaluating human angiotensinogen levels.[12] We found elevated urinary angiotensinogen levels in patients with chronic kidney disease, including patients with diabetes.[13] On the basis of these findings, we hypothesize that urinary angiotensinogen levels can function as early biomarkers for determining the status of the intrarenal RAS in normoalbuminuric patients with type 1 diabetes. Therefore, the aim of this study was to demonstrate that urinary angiotensinogen levels increased before the onset of microalbuminuria in patients with type 1 diabetes compared with age- and sex-matched control subjects.

METHODS

Study Design and Sample Collections

The experimental protocol for this study was approved by the institutional review board at Tulane University and Tokushima University. We recruited 55 subjects (34 patients with type 1 diabetes and 21 controls) at the Tokushima University Hospital and its associated clinics. All samples were obtained after obtaining written informed consent. Because the primary focus of the study was comparison between characteristics of normoalbuminuric patients with type 1 diabetes and those of control subjects, 6 microalbuminuric patients with type 1 diabetes (urinary albumin:creatinine ratio >30 mg/g) were excluded. Consequently, 49 urine and plasma samples were analyzed. No subject received angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. A random spot urine sample and a blood sample were obtained from all participants. Height, body weight, systolic blood pressure, and diastolic blood pressure were recorded on the same day.

Measurements

Urinary sodium and protein concentrations were determined in the clinical laboratory of the Tokushima University Hospital. Urinary albumin and creatinine concentrations were measured using an automated analyzer (Bayer). Urinary and plasma concentrations of angiotensinogen were measured using commercially available ELISA kits (IBL), as previously described.[14] Serum cystatin C levels were measured using a commercially available ELISA kit (BioVendor), and estimated glomerular filtration rate (eGFR) was calculated as [100/serum cystatin C], because serum eGFR based on cystatin C levels is more accurate than that based on creatinine levels, especially in patients with type 1 diabetes.[15]

Statistical Analyses

Pearson single regression analyses and Spearman single regression analyses were performed for statistical analysis of the parametric and nonparametric data, respectively. Unpaired *t* test was performed to determine the group means. All data were presented as means ± SEM. $P < 0.05$ was considered significant. All computations, including data management and statistical analyses, were performed using JMP software (SAS Institute).

RESULTS

Subjects Profiles and Laboratory Data

The demographics and the baseline laboratory data of the included subjects are summarized in Table 1 and Table 2.

Single Regression Analyses

The urinary angiotensinogen:creatinine ratio was significantly positively correlated with the urinary protein:creatinine ratio ($r = 0.37$, $P = 0.0093$). However, the urinary angiotensinogen:creatinine ratio was not correlated with sex, age, height ($P = 0.7309$), body weight ($P = 0.2556$), body mass index ($P = 0.1007$), systolic blood pressure ($P = 0.9834$), diastolic blood pressure ($P = 0.7946$), urinary sodium:creatinine ratio, urinary albumin:creatinine ratio, plasma angiotensinogen levels, or eGFR.

Urinary Angiotensinogen Levels in Patients With Type 1 Diabetes

The urinary angiotensinogen:creatinine ratio was significantly greater in patients with type 1 diabetes than in control subjects (Table 2). Importantly, an increase in plasma angiotensinogen levels was not observed in plasma (Table 2).

DISCUSSION

The urinary angiotensinogen:creatinine ratio was compared between patients with type 1 diabetes and control subjects. Neither urinary albumin:creatinine ratio nor urinary protein: creatinine ratio increased in patients with type 1 diabetes compared with control subjects, suggesting that these patients were in their premicroalbuminuric phase of diabetic nephropathy. However, the urinary angiotensinogen:creatinine ratio was significantly higher in patients with type 1 diabetes than in control subjects. In particular, an increase in plasma angiotensinogen levels was not observed. These data indicate that in patients with type 1 diabetes, the urinary angiotensinogen levels increase and that this increase is precedent to increased urinary albumin levels. This suggests that urinary angiotensinogen levels may serve as highly sensitive markers for determining the activation of the intrarenal RAS; consequently, they can also serve as early markers of diabetic nephropathy.

In this study, subjects were randomly recruited, without any bias in the selection process. As a result, there were some deviations in the grouping of height, body weight, body mass index, systolic blood pressure, and diastolic blood pressure (Table 1). However, none of these parameters correlated with the urinary angiotensinogen:creatinine ratio. Therefore, it seems unlikely that these differences affect the final results reported herein.

Although most of the circulating angiotensinogen is produced and secreted by the liver, the kidneys also produce angiotensinogen.[7] Intrarenal angiotensinogen mRNA and protein have been localized to proximal tubule cells, indicating that the intratubular angiotensin II could be derived from locally formed and secreted angiotensinogen.[16,17] The angiotensinogen produced in proximal tubule cells seems to be secreted directly into the tubular lumen in addition to producing its metabolites intracellularly and secreting them into the tubular lumen.[18] Proximal

tubular angiotensinogen concentrations in anesthetized rats have been reported in the range of 300 to 600 nmol/L, which greatly exceed the free angiotensin I and angiotensin II tubular fluid concentrations.[5] Because of its molecular size (50–60 kDa), little plasma angiotensinogen is expected to filter across the glomerular membrane, further supporting the concept that proximal tubular cells secrete angiotensinogen directly into the tubules.[19] To determine whether circulating angiotensinogen is a source of urinary angiotensinogen, human angiotensinogen was infused into hypertensive and normotensive rats, and it was found that circulating human angiotensinogen was not detectable in the urine.[20] The failure to detect human angiotensinogen in the urine indicates limited glomerular permeability and/or tubular degradation. These findings support the hypothesis that urinary angiotensinogen comes from the angiotensinogen that is formed and secreted by the proximal tubules and not from plasma. In agreement with this concept, plasma angiotensinogen levels were not correlated with the urinary angiotensinogen:creatinine ratio in this study. Moreover, plasma angiotensinogen levels were not different between the 2 groups even though urinary angiotensinogen:creatinine ratio was significantly different between the 2 groups in this study. Therefore, it seems highly likely that angiotensinogen in urine originates from angiotensinogen in kidney, not from angiotensinogen in plasma.

The relatively small sample size in this study is a potential limitation. However, our observation demonstrates that the levels of urinary angiotensinogen increase in patients with type 1 diabetes, even before diagnosis of proteinuria or microalbuminuria. A larger multicenter, randomized, control study is necessary to extend these observations for their clinical applicability. On the basis of these findings, a randomized clinical trial is under preparation.

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TABLE 1

Subject profiles

Parameters	Control subjects, N = 21	Patients with type 1 diabetes, N = 28	*P*	χ^2
Sex (female/ male)	10/11	17/11	0.3618	0.832
Age (yr)	16.7 ± 1.6	17.4 ± 1.0	0.6870	
Height (cm)	148.0 ± 4.6	158.1 ± 1.8[a]	0.0230	
Body weight (kg)	44.4 ± 3.7	57.0 ± 2.1[a]	0.0029	
Body mass index (kg/m^2)	19.0 ± 1.0	22.6 ± 0.6[a]	0.0019	
Systolic BP (mm Hg)	111.4 ± 1.3	122.9 ± 2.5[a]	0.0001	
Diastolic BP (mm Hg)	61.3 ± 1.5	72.5 ± 2.8[a]	0.0007	

[a] $P < 0.05$ vs. control subjects.

BP, blood pressure.

TABLE 2

Laboratory data

Parameters	Control subjects, N = 21	Patients with type 1 diabetes, N = 28	*P*
Urinary sodium: creatinine ratio (mEq/g)	138.7 ± 24.2	146.4 ± 19.6	0.8138
Urinary protein: creatinine ratio (g/g)	0.07 ± 0.01	0.06 ± 0.01	0.3231
Urinary albumin: creatinine ratio (mg/g)	8.5 ± 1.1	8.8 ± 0.7	0.8450
eGFR (mL/min/ 1.73 m^2)	122.4 ± 9.3	135.8 ± 5.1	0.3100
Plasma angiotensinogen (μg/mL)	29.5 ± 3.3	26.3 ± 1.3	0.3148
Urinary angiotensinogen: creatinine ratio (μg/g)	4.2 ± 0.7	12.1 ± 3.2[a]	0.0454

[a] $P < 0.05$ vs. control subjects.

eGFR, estimated glomerular filtration rate.