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Original Article

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Urinary angiotensinogen as a potential biomarker of diabetic nephropathy

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Abstract

Background. Activation of the renin–angiotensin–aldosterone system (RAAS) is an important mediator of diabetic nephropathy. Urinary angiotensinogen, a novel biomarker of the intrarenal RAAS, is associated with progressive kidney injury. In this study, the authors investigated the determinants of urinary angiotensinogen and its associations with staging of diabetic nephropathy.

Methods. Random urine samples were collected from the patients with type 2 diabetes with normoalbuminuria ($n = 52$), microalbuminuria ($n = 52$) and macroalbuminuria ($n = 51$) for the measurement of angiotensinogen by sensitive and specific ELISAs. Control samples were collected from healthy volunteers ($n = 20$) who had normal albuminuria and renal function.

Results. Urinary angiotensinogen was higher in microalbuminuric and macroalbuminuric diabetes than in controls [63.44 (interquartile range, IQR: 22.08, 174.8) versus 398.38 (IQR: 205.03, 673.68) versus 9.12 (IQR: 3.76, 23.82) ng/mg creatinine, respectively, $P < 0.001$]. In diabetes with normoalbuminuria, urinary angiotensinogen was also higher than in controls [16.42 (IQR: 7.69, 34.71) versus 9.12 (IQR: 3.76, 23.82) ng/mg creatinine, $P = 0.047$]. The performance of the biomarker in differentiating each stage of type 2 diabetes from controls was illustrated by receiver-operating characteristic curves. The areas under the curve for the diagnosis of established normoalbuminuric, microalbuminuric and macroalbuminuric type 2 diabetes using urine angiotensinogen (ng/mg creatinine) were 0.62 (95% CI: 0.48–0.77), 0.85 (95% CI: 0.76–0.94) and 0.96 (95% CI: 0.92–1.00), respectively. In addition, the cut-off levels were 9.30 ng/mg (sensitivity 65.4%, specificity 55.0%), 12.32 ng/mg (sensitivity 55.8%, specificity 65.0%) and 17.44 ng/mg (sensitivity 44.2%, specificity 70.0%), respectively, for distinguishing normoalbuminuric type 2 diabetes from healthy controls.

Conclusions. The authors propose that angiotensinogen could be one of the potential urinary biomarkers for diagnosis in established diabetic nephropathy. It appeared even before the significant albuminuria in diabetic nephropathy. It might be useful as an early biomarker of activation of the renin–angiotensin system in diabetic nephropathy.

Keywords: biomarker; diabetic nephropathy; renin–angiotensin–aldosterone system; urinary angiotensinogen

Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD), representing 30–47% of the United States and Asian populations undergoing long-term dialysis [1, 2]. Intensive insulin therapy and blood pressure control with renin–angiotensin system (RAS) blockers are effective in retarding diabetic nephropathy progression [3]. Effective and early diagnosis is a critical concern.

Accurately assessing and monitoring renal function is of critical importance in patients with diabetic nephropathy. Urinary albumin has been recognized as the 'gold standard' marker of glomerular injury in diabetic patients; however, one-third of patients with diabetic nephropathy lost renal function even during the normoalbuminuric and microalbuminuric stages. In addition, frequent regression of microalbuminuria in patients with type 1 diabetes indicates that elevated urinary albumin excretion does not imply inexorably progressive nephropathy [4]. Thus, more sensitive biomarkers and a specific biomarker for diabetic nephropathy are needed.

RAS activation plays an important role, being present in the early stages of diabetic nephropathy and then being exacerbated by albumin leakage from glomerular capillaries, overproduction of mesangial cell matrix, podocyte injury and proximal tubular injury [5]. Therefore, activated RAS markers might be useful biomarkers for the diagnosis or monitoring of diabetic complications, particularly kidney disease. Recent evidence based on experimental animal models shows that enhanced intrarenal angiotensinogen formation during angiotensin II infusion is reflected by secretion into the tubular fluid leading to increased urinary angiotensinogen [6, 7]. The presence of

angiotensinogen in the urine in kidney disease has been known for many years [8]; however, few reports have addressed the urinary angiotensinogen in patients with diabetic kidney injury [9–11]. The aim of this study was to investigate the determinants of urinary angiotensinogen and their associations with staging of diabetic nephropathy.

Materials and methods

Subjects with type 2 diabetes were recruited from our outpatient nephrology clinic. Random urine samples were collected from the patients with normoalbuminuria, microalbuminuria and macroalbuminuria and stored at −80°C until assayed. Control samples were collected from healthy volunteers with normal renal function and normoalbuminuria. Inclusion criteria of the study included age, 18 years or older, and patients with type 2 diabetes. Exclusion criteria included active malignancy, severe heart, lung or liver disease, stroke, chronic infection, e.g. tuberculosis within 1 year of starting the study, and any immunological or inflammatory disorders. A complete medical history and physical examination were performed on all subjects. All subjects fasted for at least 12 h overnight before all blood drawing. Complete blood counts, blood urea nitrogen (BUN), serum creatinine and comprehensive serum chemistries were measured. The serum concentration of creatinine was measured using the enzymatic method. The glomerular filtration rate (GFR) was estimated from calibrated serum creatinine with the 2009 CKD-EPI creatinine equation [12]. The study was approved by the Institutional Review Boards of the Royal Thai Army Medical Department, Bangkok, Thailand. All participants gave their written and informed consent.

Urinary biomarker for kidney injury

Urinary albumin and creatinine concentrations were measured and expressed as the urinary albumin creatinine ratio (UACR). Diabetic nephropathy status was determined by measuring UACR in at least two of the last three urine specimens. Patients were divided into three groups according to levels of UACR and urine protein: normoalbuminuria (UACR < 30 mg albumin/g creatinine), microalbuminuria (UACR 30–300 mg albumin/g creatinine) and overt nephropathy (UACR >300 mg albumin/g creatinine and/or persistent proteinuria).

The urine angiotensinogen was performed using a commercially available ELISA assay (Angiotensinogen ELISA JP27412 Kit; Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan) that specifically detects human angiotensinogen in serum, urine and cell culture supernatants. The assay was performed according to the manufacturer's protocols.

Statistical analysis

Data are given as mean ± SD for continuous variables or as a percentage in categorical variables. Statistical analysis was performed using SPSS, version 15. Either the two-sample *t*-test or Mann–Whitney rank-sum test was used for continuous variables. For multiple comparisons, ANOVA was used followed by the least significance difference test. Spearman correlation coefficients were used as appropriate to test correlations between urine angiotensinogen and other variables. A multivariate model using a stepwise regression analysis was performed to correct for confounders. Receiver-operating characteristics (ROC) analysis was used to calculate the area under the curve (AUC) for angiotensinogen and to find the best cut-off values for identifying diabetic nephropathy. A $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Subjects with type 2 diabetes with normoalbuminuria ($n = 52$), microalbuminuria ($n = 52$) and macroalbuminuria ($n = 51$) and healthy volunteers ($n = 20$) were enrolled in the study. Baseline characteristics are shown in Table 1. Age, gender, body weight, systolic blood pressure, fasting plasma glucose, haemoglobin A1C, BUN, serum creatinine, estimated GFR, UACR, cholesterol and triglycerides were different between patients with type 2 diabetes and controls (Table 1).

Increased urine excretion of angiotensinogen in type 2 diabetes

The urine angiotensinogen level in healthy controls [7.42 (IQR: 3.47, 12.74) ng/mL] was significantly less than in patients with normoalbuminuric type 2 diabetes [12.79 (IQR: 5.47, 26.63) ng/mL, $P = 0.048$], microalbuminuric type 2 diabetes [39 (IQR: 11.89, 86.16) ng/mL, $P < 0.001$] and macroalbuminuric type 2 diabetes [306.42 (IQR: 138.21, 388.21) ng/mL, $P < 0.001$] (Figure 1A). In addition, significant differences were found between the urine angiotensinogen values in each stage of diabetic nephropathy. Meanwhile, urinary angiotensinogen and creatinine ratio (UAngCR) was clearly detectable in the patients with normoalbuminuric, microalbuminuric and macroalbuminuric type 2 diabetes than in healthy controls [16.42 (IQR: 7.69, 34.71), 63.44 (IQR: 22.08, 174.8) and 398.38 (IQR: 205.03, 673.68) versus 9.12 (IQR: 3.76, 23.82) ng/mg creatinine, respectively, $P < 0.001$] (Figure 1B). The appearance of urine angiotensinogen in patients with type 2 diabetes but not in healthy controls underscores its value as a potential biomarker for kidney injury in albuminuric and non-albuminuric conditions.

Initial results suggested that urine angiotensinogen reflected the intrarenal RAS activation in advanced stage of diabetic nephropathy, but urinary angiotensinogen might be a marker of glomerular filtration barrier damage. We also measured plasma angiotensinogen in all subjects. The plasma angiotensinogen levels were not significantly different in normal subjects and each stage of patients with type 2 diabetic nephropathy (Table 1).

Correlation of urinary angiotensinogen with other renal injury parameters

To assess the relationship between urine angiotensinogen and renal severity, the Spearman correlation analysis was performed as appropriate. Urine excretion of angiotensinogen, evaluated by ELISA, was plotted against urine albumin (Figure 2), estimated GFR (Figure 2) and systolic blood pressure levels (Figure 2). Urine angiotensinogen levels were not correlated with age, body weight, diastolic blood pressure, fasting plasma glucose, haemoglobin A1C and lipid profiles. However, urine angiotensinogen levels were positively correlated with significance for duration of diabetes, systolic blood pressure, BUN, serum creatinine

Table 1. Baseline characteristics of subjects

	Controls (*N* = 20)	Normoalbuminuria (*N* = 52)	Microalbuminuria (*N* = 52)	Macroalbuminuria (*N* = 51)
Male	3 (15%)	36 (69.2%)	20 (38.5%)	28 (54.9%)
Duration of diabetes (years)	–	11.35 ± 6.63	10.63 ± 4.56	13.00 ± 6.63*
Age	46.2 ± 6.43	64.67 ± 9.42**	67.69 ± 12.69**	64.94 ± 9.46**
Weight (kg)	59.92 ± 8.95	69.11 ± 15.66**	66.85 ± 12.6**	66.84 ± 12.07**
SBP (mmHg)	116.55 ± 10.57	127.88 ± 15.11**	131.63 ± 17.76**	138.74 ± 15.18*,**,***
DBP (mmHg)	72.50 ± 9.12	73.40 ± 10.99	71.73 ± 12.00	72.72 ± 10.84
Hypertension (*N*, %)	–	48 (92.3%)	48 (92.3%)	49 (96.1%)
Dyslipidemia (*N*, %)	9 (45%)	49 (94.2%)	51 (98.1%)	50 (98%)
Current medications (*N*, %)				
ACEI or ARB	–	33 (63.5%)	34 (65.4%)	31 (60.8%)
CCB	–	29 (55.8%)	39 (75.0%)	31 (60.8%)
Beta blocker	–	22 (42.3%)	20 (38.5%)	21 (41.2%)
Alpha blocker	–	8 (15.4%)	7 (13.5%)	15 (29.4%)
Diuretics	–	12 (23.1%)	18 (34.6%)	33 (64.7%)
Statin	–	50 (96.2%)	49 (94.2%)	48 (94.1%)
Laboratory data				
Plasma angiotensinogen (μg/mL)	44.11 ± 10.51	42.66 ± 10.89	39.43 ± 7.95	38.36 ± 10.79
Fasting plasma glucose (mg/dL)	78.80 ± 12.44	152.31 ± 60.57**	156.06 ± 63.31**	156.29 ± 68.29**
HbA1C (%)	5.5 ± 1.34	7.54 ± 2.08**	7.52 ± 1.8**	8.1 ± 2.29**
BUN (mg/dL)	12.09 ± 3.12	16.57 ± 6.22**	18.36 ± 9.69**	35.1 ± 18.99*,**,***
Serum creatinine (mg/dL)	0.7 (0.6, 0.8)	1 (0.8, 1.2)**	1 (0.8, 1.3)**	2.82 (2.3, 1.6)*,**,***
Cholesterol (mg/dL)	224.42 ± 30.81	154.65 ± 25.73**	169.94 ± 43.34**,***	200.53 ± 67.25*,***
Triglycerides (mg/dL)	68 (49, 104)	101 (72, 156)**	149 (91, 185)**,***	162.3 (145, 108)**,***
GFR-CKD-EPI (mL/min/1.73 m^2	97.08 (88.44, 113.65)	73.14 (61.82, 86.27)**	66.38 (44.11, 93.12)**	30.89 (28.41, 16.39)*,**,***
UACR (mg/g creatinine)	6.2 (2.2, 9.4)	6.1 (2.7, 11.5)	66.85 (42.05, 150.4)***	357.6 (357.6, 256.5)*,**,***

Data are mean ± SD, median with interquartile range and percentages; *P < 0.05 versus microalbuminuria. **P < 0.05 versus controls, ***P < 0.05 versus normoalbuminuria.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin type 1 receptor blocker; CCB, calcium channel blocker; DBP, diastolic blood pressure; HbA1C, hemoglobinA1C; SBP, systolic blood pressure; UACR, urine albumin creatinine ratio.

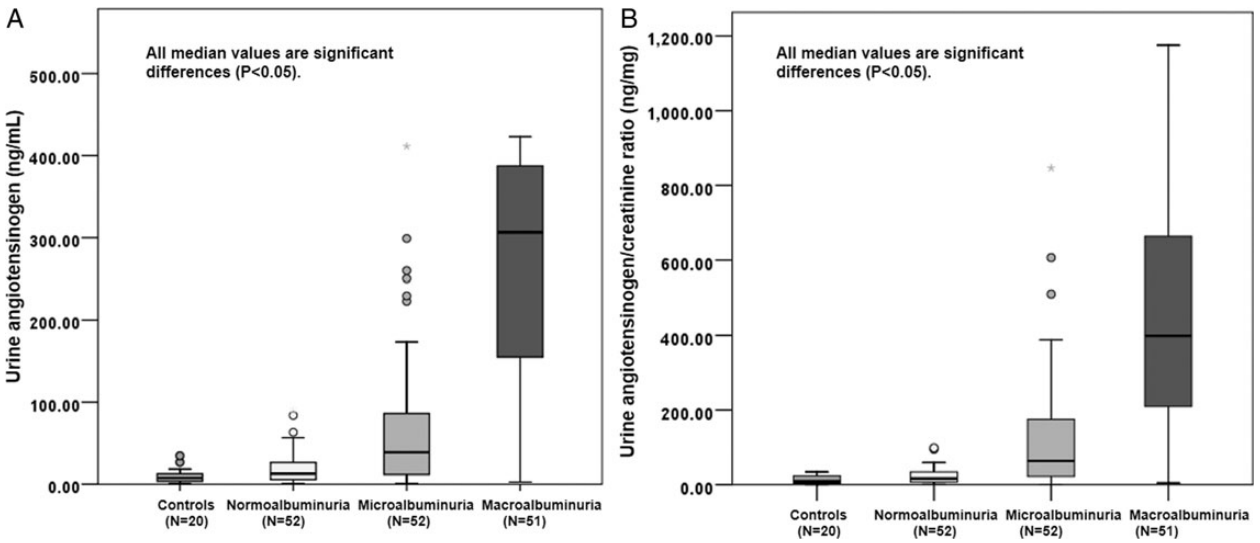

Fig. 1. Urine angiotensinogen levels in patients with diabetic nephropathy and in healthy controls. There was a significant difference between the urine angiotensinogen values in the each stage of diabetic nephropathy (P < 0.05). Data are median with interquartile range.

and UACR, but negatively correlated with estimated GFR (Tables 2 and 3). After multiple regression analyses, only significant correlations were found between urine albumin and increasing urine angiotensinogen levels (Tables 2 and 3). In general, the increase in urine albumin, BUN and serum creatinine and the decrease in estimated GFR represent severity or aggravated renal function in patients with diabetic nephropathy. These data are consistent with the hypothesis that urinary angiotensinogen levels were associated with severity or exaggerated renal function in patients with type 2 diabetes.

Performance of urinary angiotensinogen in diagnosing diabetic nephropathy

The ROC analysis of urine angiotensinogen (ng/mL) and UAngCR (ng/mg creatinine) in diagnosing each stage of diabetic nephropathy is illustrated in Table 4. The AUC-for the diagnosis of established normoalbuminuric, microalbuminuric and macroalbuminuric type 2 diabetes using urine angiotensinogen (ng/mL) were 0.65 (95% CI: 0.52–0.79), 0.82 (95% CI: 0.72–0.91) and 0.95 (95% CI: 0.90–1.00), respectively. The cut-off levels were 7.84 ng/mL (sensitivity 61.5%, specificity 55.0%), 11.05 ng/mL

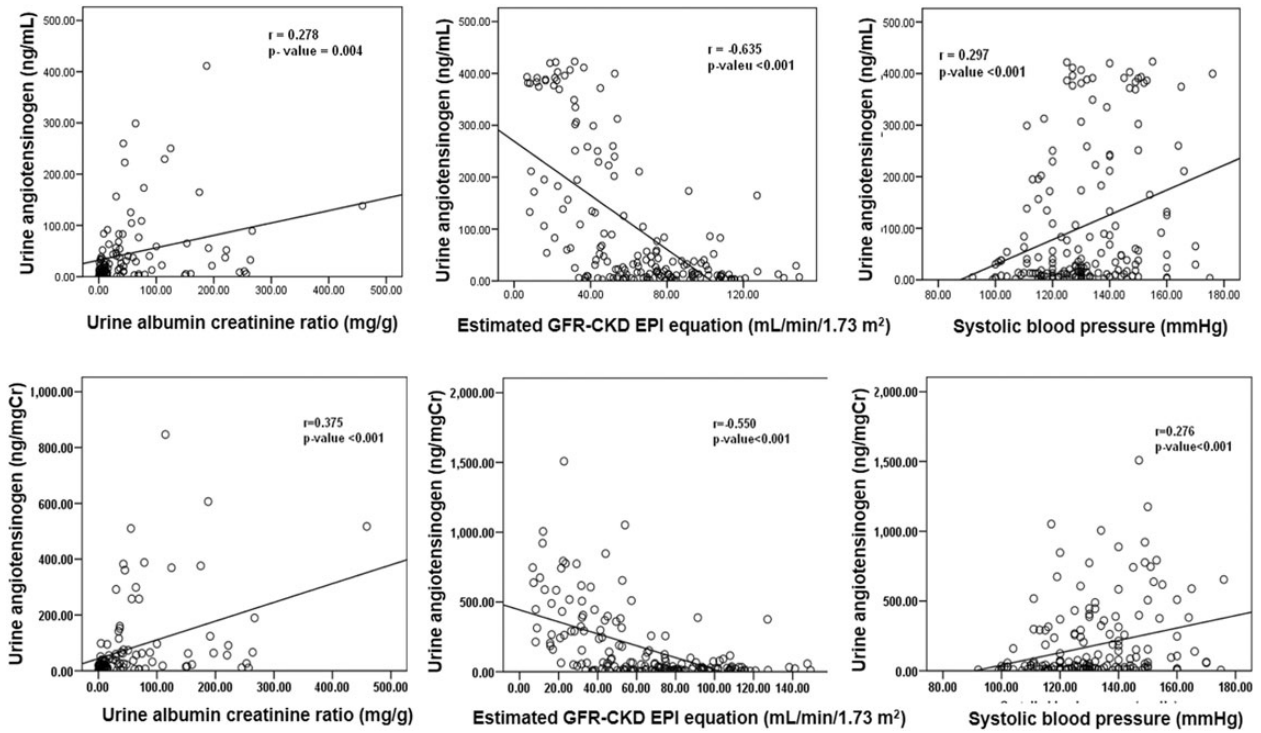

Fig. 2. Urine angiotensinogen levels were associated with severity renal function and hypertension in patients with type 2 diabetes. (A) Single regression analyses for urine angiotensinogen levels (ng/mL) with urine albumin ($r = 0.278$, P = 0.004), estimated GFR ($r = -0.635$, P < 0.001) and systolic blood pressure ($r = 0.297$, P < 0.001). (B) Single regression analyses for urine angiotensinogen levels (ng/mg creatinine) with urine albumin ($r = 0.375$, P < 0.001), estimated GFR ($r = -0.550$, P < 0.001) and systolic blood pressure ($r = 0.276$, P < 0.001).

Table 2. Univariate and multivariate regression analyses demonstrating factors showing correlation with urinary angiotensinogen levels (ng/mL)

	Univariate		Multivariate	
	R	P value	Standardized coefficient	P value
Duration of diabetes (years)	0.242	0.002	−0.21	0.852
Age (year)	0.171	0.054	–	–
Body weight (kg)	0.048	0.534	–	–
SBP (mmHg)	0.297	<0.001	0.54	0.177
DBP (mmHg)	−0.049	0.523	–	–
Fasting plasma glucose (mg/dL)	0.098	0.203	–	–
HbA1C (%)	0.105	0.214	–	–
Cholesterol (mg/dL)	0.112	0.148	–	–
Triglycerides (mg/dL)	0.145	0.060	–	–
BUN (mg/dL)	0.530	<0.001	0.64	0.496
Serum creatinine (mg/dL)	0.642	<0.001	–	–
GFR-CKD-EPI (mL/min/1.73 m^2)	−0.635	<0.001	−0.55	0.065
UACR (mg/g)	0.278	0.004	0.23	0.003

Independent variables in the multivariate model were chosen using a stepwise regression analysis where all significant variables listed in the univariate analysis were included.
DBP, diastolic blood pressure; HbA1C, hemoglobinA1C; GFR, glomerular filtration rate; SBP, systolic blood pressure; UACR, urine albumin creatinine ratio.

Table 3. Univariate and multivariate regression analyses demonstrating factors showing correlation with the urine angiotensinogen/creatinine ratio (ng/mg)

	Univariate		Multivariate	
	R	P value	Standardized coefficient	P value
Duration of diabetes (years)	0.307	<0.001	3.12	0.216
Age (year)	0.116	0.127	–	–
Body weight (kg)	0.01	0.899	–	–
SBP (mmHg)	0.276	<0.001	1.19	0.172
DBP (mmHg)	−0.029	0.703	–	–
Fasting plasma glucose (mg/dL)	0.202	0.008	–	–
HbA1C (%)	0.126	0.135	–	–
Cholesterol (mg/dL)	0.097	0.211	–	–
Triglycerides (mg/dL)	0.168	0.059	–	–
BUN (mg/dL)	0.471	<0.001	0.47	0.817
Serum creatinine (mg/dL)	0.559	<0.001	–	–
GFR-CKD-EPI (mL/min/1.73 m^2)	−0.550	<0.001	−0.78	0.226
UACR (mg/g)	0.375	<0.001	0.63	<0.001

Independent variables in the multivariate model were chosen using a stepwise regression analysis where all significant variables listed in the univariate analysis were included.
DBP, diastolic blood pressure; HbA1C, hemoglobinA1C; GFR, glomerular filtration rate; SBP, systolic blood pressure; UACR, urine albumin creatinine ratio.

(sensitivity 55.8%, specificity 65.0%) and 13.32 ng/mL (sensitivity 46.2%, specificity 80.0%) for distinguishing normoalbuminuric type 2 diabetes from healthy controls. In addition, the cut-off levels of urine angiotensinogen for diagnosing microalbuminuric and macroalbuminuric type 2 diabetes are also demonstrated in Table 4.

The AUC of UAngCR were 0.62 (95% CI: 0.48–0.77), 0.85 (95% CI: 0.76–0.94) and 0.96 (95% CI: 0.92–1.00) for diagnosis of type 2 diabetes with normoalbuminuria, microalbuminuria and macroalbuminuria, respectively. For UAngCR, the cut-off levels were 9.30 ng/mg (sensitivity

Table 4. Performance of urinary angiotensinogen in diagnosing diabetic nephropathy

Group	Cut-off value	Sensitivity (%)	Specificity (%)	Accuracy (%)	Area	Asymptotic 95% CI	
						Upper	Lower
Urine angiotensinogen (ng/mL)							
Normoalbuminuria	7.84	61.5	55.0	78.0	0.65	0.52	0.79
	11.05	55.8	65.0	58.3			
	13.32	46.2	80.0	55.6			
Microalbuminuria	11.63	75.0	65.0	72.2	0.82	0.72	0.91
	14.84	71.2	80.0	73.6			
	19.21	69.2	90.0	75.0			
Macroalbuminuria	12.74	92.2	75.0	87.3	0.95	0.90	1.00
	17.47	92.2	85.0	90.1			
	41.11	92.2	100	94.4			
UAngCR (ng/mg creatinine)							
Normoalbuminuria	9.30	65.4	55.0	62.5	0.62	0.48	0.77
	12.32	55.8	65.0	58.3			
	17.44	44.2	70.0	51.4			
Microalbuminuria	10.46	88.5	60.0	80.6	0.85	0.76	0.94
	20.28	78.8	75.0	89.1			
	33.57	65.4	95.0	73.6			
Macroalbuminuria	13.99	92.2	65.0	84.5	0.96	0.92	1.00
	28.0	92.2	80.0	88.7			
	42.1	92.2	100	94.4			

Table 5. Urine angiotensinogen levels in patients with diabetic nephropathy with and without RAS blockers

	RAS blockers (ACEI/ARB) (*N* = 98)	Non-RAS blockers (*N* = 77)	P value
Urine angiotensinogen (ng/mL)			
Normoalbuminuria	11.16 (5.47, 23.16)	12.84 (5.47, 28.21)	0.992
Microalbuminuria	31.74 (9.68, 76.74)	50.58 (21.47, 108.84)	0.229
Macroalbuminuria	312.42 (138.21, 388.21)	254.89 (153.16, 388.37)	0.862
Total	36.26 (8.84, 210.74)	26.84 (7.68, 134.53)	0.283
UAngCR (ng/mg creatinine)			
Normoalbuminuria	6.5 (4.3, 10.7)	3.8 (1.1, 15.2)	0.655
Microalbuminuria	69.4 (42.9, 160)	48 (34.4, 88.0)	0.069
Macroalbuminuria	357.6 (256.5, 458.7)	314.89 (143.1, 388.4)	0.233
Total	34.9 (6.6, 80.0)	29.6 (3.8, 45.2)	0.233

Data are median with interquartile range.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin type 1 receptor blocker.

65.4%, specificity 55.0%), 12.32 ng/mg (sensitivity 55.8%, specificity 65.0%) and 17.44 ng/mg (sensitivity 44.2%, specificity 70.0%) for distinguishing normoalbuminuric type 2 diabetes from healthy controls. The cut-off levels of UAngCR for diagnosing microalbuminuric and macroalbuminuric type 2 diabetes are also presented in Table 4. Therefore, urine angiotensinogen ELISA demonstrated moderate to high sensitivity and specificity for diagnosing diabetic nephropathy.

It is clear that patients with diabetic nephropathy benefit from angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blocker (ARBs) therapy. When patients were stratified according to their baseline anti hypertensive agents, among the subgroup of patients with and without RAS blockers, no significant difference of urine angiotensinogen levels was seen across all patients with and without RAS blockers (Table 5).

Discussion

This study describes the rising urine excretion of angiotensinogen in patients with type 2 diabetes. Urinary angiotensinogen levels correlated with albuminuria and inversely with estimated GFR. In urine, angiotensinogen ELISA demonstrated moderate to high sensitivity and specificity for diagnosing diabetic nephropathy. The following novel findings emerged: urinary angiotensinogen is increased in normoalbuminuric type 2 diabetes compared with healthy controls. Taken together, these data demonstrate that urine angiotensinogen is a likely marker of renal tubular injury and a promising urinary biomarker for early stages of disease in patients with type 2 diabetes.

The intrarenal RAS plays an important role in the progression of diabetic nephropathy [3]. The only known substrate of renin is angiotensinogen, and the changes in the level of angiotensinogen can control the activity of the RAS [13]. Enhanced intrarenal angiotensinogen levels have also been observed in multiple experimental models of hypertension [14, 15] and kidney diseases including diabetic nephropathy [16, 17]. Moreover, mice overexpressing angiotensinogen in renal proximal tubular cells develop albuminuria and intrarenal RAS activation act in concert to increase tubular apoptosis in diabetes [18]. The data suggest that the de novo expression of angiotensinogen during injury and its excretion in urine may be common events during progressive renal functional decline. In contrast, one report showed that reductions in urinary angiotensinogen paralleled the changes in albuminuria, and the urine/plasma concentration ratio of angiotensinogen was identical to that of albumin under all conditions [19]. They suggested that urinary angiotensinogen might be a marker of filtration barrier damage rather than

intrarenal RAS activity. However, in this study, urine excretion of angiotensinogen showed a high correlation with levels of albuminuria and plasma angiotensinogen concentration did not differ in each stage of diabetic nephropathy. This finding was similar to previous studies demonstrating that higher levels of urinary angiotensinogen in type 2 diabetes patients with albuminuria was a high risk factor for worsening renal function [11], and the presence of higher urinary angiotensinogen levels in patients with chronic kidney disease had increased risk of renal impairment [20]. Compared with previous data, only a small number of studies have been conducted about the urine angiotensinogen in different stages of diabetic nephropathy. Therefore, urinary excretion of angiotensinogen might be an index of intrarenal angiotensin activity and is associated with the deterioration of renal function and stages in patients with diabetic nephropathy.

In fact, tubule-interstitial injuries are closely associated with loss of renal function and appear to be better predictors of renal disease progression than glomerular damage [21]. Several patients with diabetic nephropathy lost renal function even during the normoalbuminuric and microalbuminuric stages. Thus, a more sensitive and specific marker for renal injury in diabetic nephropathy is needed. In animal kidney disease models, kidney and urinary angiotensinogen levels were significantly higher in diabetic rats before the development of diabetic nephropathy [22]. Moreover, urine angiotensinogen excretion was increased at an earlier time point before detecting urine albumin in type 1 diabetes mice induced by a single intraperitoneal injection of streptozotocin [23]. Our study demonstrated that urinary angiotensinogen levels were significantly increased in the setting of patients before the onset of microalbuminuria. This finding is congruent with previous reports demonstrating that an increase in urinary angiotensinogen levels was higher in normoalbuminuric patients with type 1 diabetes compared with control subjects [9] and that urinary angiotensinogen was also significantly increased in childhood patients with premicroalbuminuric diabetic nephropathy [24]. Therefore, urinary angiotensinogen might prove to be a sensitive biomarker in the detection of renal tubular injury of incipient nephropathy.

A shortcoming of this research was the exclusion of a long-term study on the role of urinary angiotensinogen in the development and progression of ESRD. Here, the authors focused on the increase of urinary angiotensinogen in a cross-sectional study and showed that increased urinary angiotensinogen may signal the development of RAS with different stages of diabetic nephropathy. However, several long-term clinical trials have proven that ACE inhibitors or ARBs retard the progression of diabetic nephropathy [25, 26]. Moreover, the detailed mechanisms of their renoprotective effects on the development of diabetic nephropathy might suppress the activated intrarenal angiotensinogen in the patients with type 2 diabetes. In hypertensive patients with type 2 diabetes receiving ARBs, it has been reported that the urinary angiotensinogen level and inflammatory markers were decreased [27]. Different anti-hypertensive regimens are also potential factors having different effects on the urinary angiotensinogen levels. To address this issue, we have subdivided the patients according to the anti-hypertensive regimens. However, statistically significant differences in urine angiotensinogen were not observed between the groups, likely because of the low sample numbers in each subgroup. Based on our results, we are projecting future clinical studies to focus on the level of urine angiotensinogen in the onset of nephropathy and the progression of kidney disease and the reduction of urinary angiotensinogen level by RAS inhibitors to bring about improved renal outcomes.

In summary, urinary excretion of angiotensinogen was increased significantly in type 2 diabetes patients, which was associated with an elevation in albuminuria and serum creatinine levels. The study has also demonstrated that urinary angiotensinogen levels increased before the onset of microalbuminuria and that urinary angiotensinogen can be an early biomarker of intrarenal RAS status in normoalbuminuric patients with type 2 diabetes compared with controls. Accordingly, measurements of urinary angiotensinogen levels might be a reliable means of identifying intrarenal activity of the RAS in type 2 diabetes patients.

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Conflict of interest statement. None declared.

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