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Association of urinary acidification function with the progression of diabetic kidney disease in patients with type 2 diabetes

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ABSTRACT

Objective: Although diabetic kidney disease (DKD) has been considered as a glomerulocentric disease in the past few decades, growing evidence demonstrated that tubular damage was indispensable in its pathogenesis and progression. This study was designed to investigate the association of urinary acidification dysfunction with the progression of DKD in type 2 diabetic patients.
Methods: Here the urinary acidification functions were measured from 80 participants with renal biopsy-proven DKD. The different kinds of renal tubular transportation dysfunction were analyzed, including the dysfunction of bicarbonate reabsorption, titratable acid secretion, and ammonium secretion. In addition, patients were followed up for 17 (interquartile range, 11–32) months to evaluate the effect of urinary acidification dysfunction in the progression of DKD.
Results: The most common urinary acidification dysfunction was the disorder of ammonium secretion, accounting for 53.75%. The more proteinuria excretion and the lower glomerular filtration rate (GFR) were observed in the urinary titratable acid disorder group than the normal group, and the same results were obtained for ammonium secretion disorder. Urine titratable acid was positively correlated with eGFR whereas it was inversely correlated with proteinuria, serum creatinine, and BUN. Moreover, 24 h urine protein, serum creatinine, BUN and cystatin C increased from DKD stage II to stage IV, whereas the eGFR and urine titratable acid decreased in the same way. Furthermore, Kaplan-Meier analysis and Cox regression showed that the disorder of titratable acid was an independent risk factor for DKD progression.
Conclusions: The dysfunction of urinary titratable acid is a potential biomarker for the severity of proteinuria, eGFR and glomerular lesions in patients with DKD. Moreover, the titratable acid disorder is an independent risk factor of the DKD progression.

1. Introduction

With the rising incidence of diabetes, diabetic kidney disease (DKD) has become the most common and serious diabetes complication. Diabetic kidney disease remains the primary cause of end stage renal disease (ESRD) with high morbidity and mortality[1–3]. Therefore, accurate biomarkers are needed especially for the early stages of DKD to identify patients at high risk of progression to ESRD.

Microalbuminuria is the most widely studied biomarker of DKD[4]. However, it lacks specificity and sensitivity for ESRD and progressive decline in estimated glomerular filtration rate (eGFR)[5]. Growing evidence supports the role of tubular damage in the pathogenesis and progression of DKD[6,7]. It has been established that renal tubular changes precede the onset of microalbuminuria in early DKD[8]. Rather than with glomerular sclerosis, it is believed that the prognosis of DKD correlates better with the severity of tubulointerstitial damage[9,10]. In line with this, numerous studies showed that tubular injury markers, such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and *N*-acetyl-β-d-glucosaminidase (NAG), might provide meaningful information for the prediction and prognosis of DKD[5,11].

The renal tubular transport plays a pivotal role in maintaining systemic acid-base balance through renal H^+ excretion and bicarbonate (HCO_3^-) reabsorption[12]. Renal tubular acidosis (RTA), resulted by different derangements of tubular acid transport, is a non-uremic defect of urinary acidification[13]. Based on the clinical presentation and pathophysiologic mechanism, there are four types of primary RTA[14]. Distal

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(Type I) RTA is characterized by impaired acid secretion from the collecting tubules, leading to decreased ammonium (NH_4^+) excretion, titratable acidity as well as HCO_3^- wasting[15]. Type II RTA is due to the failure of HCO_3^- reabsorption from the proximal tubule in where approximately 80% of the filtered HCO_3^- is reclaimed[16]. The type III RTA is a hybrid form of types I and II[17]. Type IV RTA is also named hyperkalemic RTA and is caused by a generalized transport disorder in the distal tubule[13]. The previous study showed that low urine pH was an independent predictor of CKD[18]. Also, it is well known that Type IV RTA is a common condition among patients with diabetes and overt nephropathy[19]. However, the relationship between RTA and DKD has not been studied.

Our study aimed to investigate the association of urinary acidification dysfunction due to RTA with the progression of DKD in type 2 diabetic patients.

2. Research design and methods

2.1. Participants

Eighty consecutive patients receiving renal biopsy and proven to have DKD between March 1, 2014 and February 28, 2018 in the department of nephrology, the First Affiliated Hospital of Nanjing Medical University, were included and followed in the present study. The exclusion criteria were as follows: (1) Patients younger than 18 years old; (2) those with other kidney diseases (such as IgA nephropathy, membranous nephropathy and so on); (3) those with severe organ insufficiency except kidney; (4) use of sodium bicarbonate tablets within 30 days. The study was approved by the ethical committee of the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from all subjects.

2.2. Measurements

All participants had demographic, clinical and laboratory data collected at recruitment and during follow up. Blood samples were taken in the morning before any food intake. Samples were centrifuged immediately and analyzed for the following parameters: serum creatinine, blood urea nitrogen (BUN), Uric acid, Cystatin C, hemoglobin, glycated hemoglobin (HbA1c), fasting blood sugar (FBG), albumin, serum lipids, PTH, 25(OH) vitamin D and electrolytes were measured at baseline in all patients, These parameters were measured according to standard methods in a routine clinical laboratory. 24 h urine sample was collected under aseptic precautions for the estimation of urinary microalbumin. Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation[20]. After three days of vegetarian diets, the urinary acidification function was detected by using a ZDJ-4B automatic potentiometric titrator (Shanghai INESA Scientific Instrument Co., Shanghai, China)[21]. In brief, the fasting morning urine (10 mL) was taken and the original urine pH was measured. 10 mL hydrochloric acid solution (0.1 mmol/L) was added to the urine sample and the solution was titrated with sodium hydroxide (NaOH) solution (0.1 mmol/L) to original urine pH. Record the volume of NaOH (V1, mL). Continue the titration with NaOH solution until pH = 7.4. Record the volume of NaOH (V2, mL). Then 10 mL formaldehyde solution (pH = 7.4) was added to the urine sample titrated with NaOH solution to pH = 7.4. Record the volume of NaOH (V3, mL). 10 mL of distilled water was taken and the original water pH was measured. Then 5 ml hydrochloric acid solution (0.1 mmol/L) was added to the water sample and the solution was titrated with NaOH solution (0.1 mmol/L) to original water pH. Record the volume of NaOH (V0, mL). $HCO3^-$ (mmol/L) = (V0-V1) × 10. TA (mmol/L) = V2 × 10. $NH4^+$ (mmol/L) = V3 × 10. Normal values of the urinary acidification function are as follows: urine pH (5.0–8.0), bicarbonate ≤30 mmol/L, titratable acid ≥ 10 mmol/L, and ammonium ≥ 20 mmol/L.

All specimens were routinely examined by light microscopy, immunofluorescence and electron microscopy. According to criteria proposed by Tervaert et al., the glomerular classification of DKD was as follows: class I, glomerular basement membrane thickening; class IIa, mild mesangial expansion (mild mesangial expansion in >25% of the observed mesangium); class IIb, severe mesangial expansion (severe mesangial expansion in >25% of the observed mesangium); class III, nodular sclerosis (at least one convincing Kimmelstiel-Wilson lesion) and class IV, global glomerulosclerosis in >50% of glomeruli[22].

2.3. Outcomes

We defined the primary outcome as a composite of ESRD (GFR < 15 mL/min/1.73 m^2 or the initiation of chronic renal replacement therapy) or doubling of baseline serum creatinine. The last follow-up data collection occurred on 15th Oct 2018 and patients who had not reached a study endpoint were censored on this date.

2.4. Statistical analysis

The data were evaluated using SPSS version 22. For continuous variables, data were presented as the mean ± standard deviation (SD). Normally distributed data were compared using the independent-samples *t*-test and the one-way analysis of variance (ANOVA). Non-normally distributed data were compared using the nonparametric test. For categorical variables, data were presented as percentages, and the differences were analyzed using the Chi-square test. Correlations between urinary acidification function and clinical characteristics were assessed using the Pearson test.

Renal outcomes were evaluated using Kaplan-Meier survival analysis, and links between renal outcomes and clinical characteristics were assessed using Cox regression. Multivariate Cox analysis was used to determine independent predictors of prognosis. $p < 0.05$ was considered to be statistically significant.

Table 1
Baseline characteristics in patients with diabetic kidney disease ($n = 80$).

Variable	Data
Male sex [n(%)]	65(81.25)
Age (years)	52.00 ± 12.18
Diabetes duration (years)	10.30 ± 6.62
SBP (mmHg)	146.24 ± 24.99
DBP (mmHg)	86.15 ± 15.78
eGFR (mL/min/1.73 m2)	62.57 ± 32.28
24 h urine protein (g/d)	4.32 ± 3.82
Hb (g/L)	109.75 ± 22.71
HbA1C (%)	7.83 ± 2.06
FBG (mmol/L)	8.21 ± 5.64
Cr (umol/L)	146.10 ± 94.16
BUN (mmol/L)	9.92 ± 5.17
Uric acid (umol/L)	389.28 ± 103.20
Total protein (g/L)	57.56 ± 8.45
Albumin (g/L)	31.97 ± 6.58
TC (mmol/L)	2.00 ± 1.82
TG (mmol/L)	5.15 ± 1.61
Cystatin C (mg/L)	2.07 ± 0.97
PTH (pg/mL)	62.24 ± 61.09
25 (OH) vitamin D (nmol/L)	27.08 ± 18.38
K (mmol/L)	4.04 ± 0.55
Ca (mmol/L)	2.13 ± 0.15
P (mmol/L)	1.26 ± 0.22
Na (mmol/L)	140.55 ± 2.55
Cl (mmol/L)	107.14 ± 3.81
Nephrotic syndrome [n(%)]	21(26.25)

Table 2
The ratio of different types of urinary acidification dysfunction in DKD patients(n = 80).

	Bicarbonate	Titratable acid	Ammonium	*P* value
Abnormal[n(%)]	3(3.75)	19(23.75)	43(53.75)	<0.001

3. Results

3.1. Baseline characteristics of patients with DKD

A total of 80 patients were enrolled and followed in the study. The baseline characteristics are shown in Table 1. There were 65 males and 15 females, aged 52.00 ± 12.18 years. The mean duration of diabetes was 10.30 ± 6.62 years. Estimated GFR was 62.57 ± 32.28 mL/min/1.73m^2 and urine protein was 4.32 ± 3.82 g/24 h.

3.2. Prevalence of urinary acidification dysfunction

Consistent with expectations, urinary acidification dysfunction was common in this sample of people with diabetic kidney disease. There were significant differences in the types of urinary acidification dysfunction ($p < 0.001$). The impairment in NH_4^+ excretion is the most common, accounting for 53.75% (Table 2).

3.3. Associations of urinary acidification function with baseline characteristics

As shown in Table 3, patients with bicarbonate reabsorption disorder were younger, had lower SBP and had high HbA1C compared to those without bicarbonate reabsorption disorder. Significantly lower levels of eGFR, Hb, Albumin, 25(OH) vitamin D and Ca^{2+} were observed in patients with titratable acid disorder, along with higher levels of 24 h urine protein, HbA1C, and serum creatinine. There were also higher levels of proteinuria, HbA1C, serum creatinine, BUN, PTH and K^+ and lower levels of eGFR and Hb in the ammonium secretion disorder group than the normal group.

The association between urinary acidification function and 24 h urine protein, eGFR, serum creatinine, and BUN were analyzed (Fig. 1). Urine pH had a significant correlation with proteinuria ($r = 0.41$, $p < 0.001$). Urine bicarbonate was negatively associated with eGFR ($r = -0.252$, $p = 0.025$). Urine titratable acid was positively correlated with eGFR ($r = 0.402$, $p < 0.001$), whereas it was inversely correlated with proteinuria ($r = -0.41$, $p < 0.001$), serum creatinine ($r = -0.327$, $p = 0.003$) and BUN ($r = -0.296$, $p = 0.008$). There were also significantly positive correlations between urine ammonium and eGFR ($r = 0.313$, $p = 0.005$), while urine ammonium was inversely correlated with serum creatinine ($r = -0.256$, $p = 0.022$) and BUN ($r = -0.293$, $p = 0.008$). No other differences were observed between urinary acidification function and those clinical features.

3.4. Associations of urinary acidification function with renal pathology

In the current study, DKD patients were divided into 4 groups according to different glomerular lesions. As shown in Table 4, there were 9, 16, 41 and 14 people in the study with DKD stages IIa, IIb, III, and IV, respectively. eGFR, 24 h urine protein, Hb, serum creatinine, BUN, total protein, albumin, TC, cystatin C, 25(OH) vitamin D, Ca^{2+}, Cl^- and urine titratable acid were found to be significantly different between the groups. Among them, 24 h urine protein, serum creatinine, BUN and cystatin C increased from DKD stage II to stage IV, whereas the eGFR, Hb, 25(OH) vitamin D, and urine titratable acid decreased in the same way. There were no differences in urine pH, titratable acid and ammonium among groups.

3.5. Associations of urinary acidification function with renal outcomes

During a median follow-up time of 17 (11–32) months, 29 patients developed ESRD or doubling of serum creatinine. Kaplan-Meier analysis showed that patients with the disorder of urine

Table 3
The baseline characteristics of DKD patients with or without the dysfunction of urinary acidification (n = 80).

	Bicarbonate			Titratable acid			Ammonium		
	≤30 mmol/L ($n = 77$)	>30 mmol/L ($n = 3$)	*P* value	>10 mmol/L ($n = 52$)	≤10 mmol/L ($n = 28$)	*P* value	>20 mmol/L ($n = 38$)	≤20 mmol/L ($n = 42$)	*P* value
Male sex [n(%)]	62(80.52)	3(100)	0.396	44(84.62)	21(75.00)	0.293	30(78.95)	35(83.33)	0.616
Age (years)	52.57 ± 11.79	37.33 ± 15.53	0.033	52.46 ± 11.40	51.14 ± 13.70	0.647	50.71 ± 11.76	53.17 ± 12.58	0.371
Diabetes duration (years)	10.35 ± 6.70	9.33 ± 4.73	0.935	9.69 ± 7.01	11.46 ± 5.76	0.218	9.09 ± 7.04	11.42 ± 6.07	0.089
SBP (mmHg)	147.47 ± 24.62	116.67 ± 14.57	0.036	142.96 ± 23.44	152.42 ± 27.06	0.119	145.89 ± 25.03	146.56 ± 25.28	0.908
DBP (mmHg)	86.68 ± 15.83	73.33 ± 7.77	0.079	85.16 ± 15.00	88.00 ± 17.32	0.64	88.72 ± 14.57	83.77 ± 16.66	0.093
eGFR (mL/min/1.73 m2)	63.11 ± 32.70	48.91 ± 16.03	0.259	67.85 ± 29.49	52.95 ± 35.38	0.049	73.81 ± 30.47	52.67 ± 30.88	0.003
24 h urine protein (g/d)	4.30 ± 3.84	5.02 ± 3.75	0.404	3.20 ± 3.17	6.37 ± 4.09	<0.001	3.35 ± 3.31	5.18 ± 4.06	0.021
Hb (g/L)	110.06 ± 23.02	101.67 ± 11.59	0.533	115.92 ± 23.28	98.29 ± 16.58	<0.001	118.26 ± 24.31	102.05 ± 18.22	0.001
HbA1C (%)	7.64 ± 1.85	12.13 ± 2.23	0.012	6.91 ± 1.29	9.47 ± 2.18	<0.001	7.00 ± 1.54	8.54 ± 2.20	0.001
FBG (mmol/L)	7.85 ± 4.12	17.64 ± 21.75	0.97	7.29 ± 3.12	9.93 ± 8.37	0.226	7.46 ± 3.26	8.89 ± 7.12	0.711
Cr (umol/L)	145.48 ± 95.72	162.23 ± 40.31	0.239	126.80 ± 71.52	181.96 ± 119.27	0.034	117.21 ± 72.60	172.25 ± 104.17	0.003
BUN (mmol/L)	9.81 ± 5.05	12.78 ± 8.63	0.604	9.06 ± 3.71	11.54 ± 6.93	0.246	8.02 ± 2.83	11.65 ± 6.15	0.006
Uric acid (umol/L)	387.87 ± 102.12	425.37 ± 149.40	0.54	381.47 ± 96.10	403.78 ± 115.67	0.388	370.49 ± 101.28	406.27 ± 103.15	0.122
Total protein (g/L)	57.58 ± 8.31	57.03 ± 13.91	0.952	58.88 ± 8.27	55.09 ± 8.38	0.055	58.05 ± 9.53	57.11 ± 7.43	0.62
Albumin (g/L)	32.05 ± 6.42	30.00 ± 11.61	0.6	33.18 ± 6.51	29.73 ± 6.21	0.024	32.32 ± 6.93	31.65 ± 6.30	0.652
TC (mmol/L)	2.02 ± 1.86	1.41 ± 0.11	0.839	2.06 ± 2.10	1.87 ± 1.21	0.377	1.77 ± 1.35	2.20 ± 2.18	0.461
TG (mmol/L)	5.10 ± 1.58	6.23 ± 2.36	0.336	5.04 ± 1.50	5.35 ± 1.81	0.675	5.29 ± 1.50	5.02 ± 1.71	0.214
Cystatin C (mg/L)	2.09 ± 0.99	1.64 ± 0.46	0.484	1.86 ± 0.86	2.37 ± 1.07	0.106	1.81 ± 0.82	2.24 ± 1.04	0.137
PTH (pg/mL)	64.01 ± 61.86	22.67 ± 5.15	0.063	52.47 ± 40.29	80.98 ± 86.39	0.344	43.58 ± 35.14	78.89 ± 73.84	0.009
25 (OH) vitamin D (nmol/L)	27.06 ± 18.62	27.59 ± 5.86	0.739	30.70 ± 20.28	19.52 ± 10.36	0.02	30.60 ± 19.88	23.92 ± 16.53	0.103
K (mmol/L)	4.03 ± 0.55	4.23 ± 0.67	0.544	4.07 ± 0.50	3.98 ± 0.65	0.544	3.88 ± 0.41	4.19 ± 0.63	0.011
Ca (mmol/L)	2.12 ± 0.15	2.23 ± 0.22	0.255	2.15 ± 0.15	2.08 ± 0.15	0.038	2.15 ± 0.13	2.11 ± 0.17	0.149
P (mmol/L)	1.26 ± 0.22	1.36 ± 0.26	0.355	1.22 ± 0.18	1.33 ± 0.27	0.138	1.21 ± 0.18	1.30 ± 0.25	0.214
Na (mmol/L)	140.66 ± 2.42	137.77 ± 4.71	0.323	140.47 ± 2.28	140.69 ± 3.03	0.631	140.65 ± 2.54	140.45 ± 2.58	0.716
Cl (mmol/L)	107.24 ± 3.52	104.50 ± 9.46	0.898	107.04 ± 3.77	107.31 ± 3.94	0.492	106.99 ± 3.92	107.27 ± 3.74	0.586

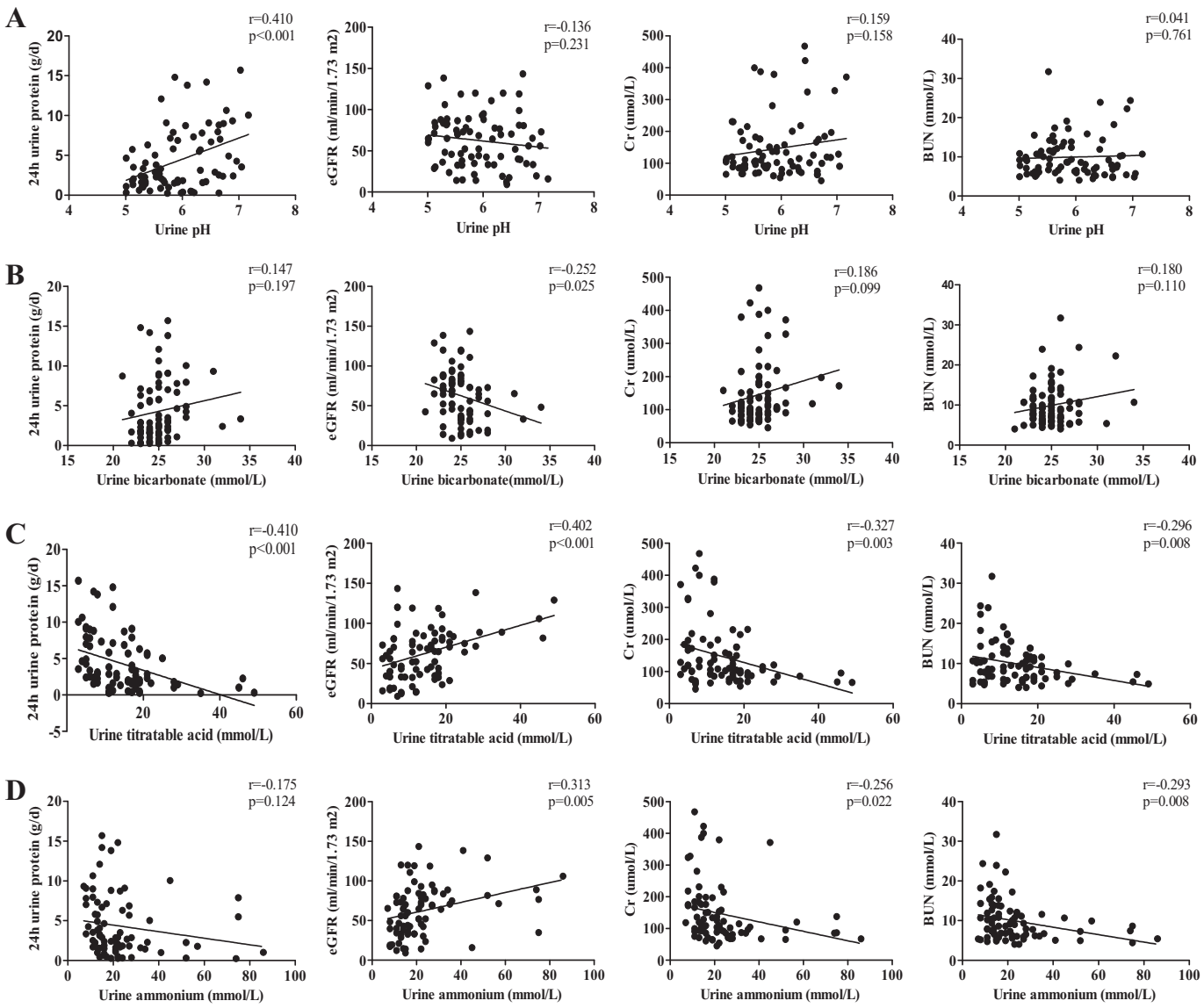

Fig. 1. Correlations between urinary acidification function and clinical characteristics. A: Correlation between urine pH and 24 h urine protein, eGFR, Cr and BUN, respectively. B: Correlation between urine bicarbonate and 24 h urine protein, eGFR, Cr and BUN, respectively. C: Correlation between urine titratable acid and 24 h urine protein, eGFR, Cr and BUN, respectively. D: Correlation between urine ammonium and 24 h urine protein, eGFR, Cr and BUN, respectively.

titratable acid at baseline had an increased risk for a renal endpoint during the follow-period ($p = 0.01$) (Fig. 2). Univariable Cox regression analysis showed that eGFR, proteinuria and the urine titratable acid disorder were risk factors for renal outcomes (Table 5). After adjustment for the baseline sex, age, blood pressure, and diabetes duration, the urine titratable acid disorder remained an independent risk factor for renal survival in multivariate Cox regression (HR 2.306, 95%CI 1.025–5.187).

4. Discussion

In this study, bicarbonate reabsorption was reduced and acid excretion was decreased in patients with DKD, suggesting the impaired urinary acidification due to a defect in the ability of the renal tubules. Especially, we found that more proteinuria excretion and lower eGFR in the titratable acid disorder group than those without titratable acid disorder. Urine titratable acid was positively correlated with eGFR whereas it was inversely correlated with proteinuria, serum creatinine, and BUN. Moreover, 24 h urine protein, serum creatinine, BUN and cystatin C increased from DKD stage II to stage IV, whereas the eGFR and urine titratable acid decreased in the same way. The urine titratable acid disorder was shown to be an independent risk factor for DKD progression to ESRD or doubling serum creatinine.

DKD remains one of the major end-organ complications of diabetes and continues to be the leading contributor to end stage renal disease[23]. Recent researches have focused on the role of tubular damage in the pathogenesis and progression of DKD[6]. Several studies have shown that tubular damage is associated with the severity of proteinuria in patients with DKD[24,25]. Our study showed that the level of proteinuria in the titratable acid excretion disorder group was significantly higher than the normal group, and the disorder of titratable acid was positively associated with the severity of proteinuria. Although there was no significant correlation between ammonium secretion dysfunction and the severity of proteinuria, more proteinuria excretion was also observed in the ammonium secretion

Table 4
Baseline characteristics of DKD patients according to pathological classification.

	IIa($n = 9$)	IIb($n = 16$)	III($n = 41$)	IV($n = 14$)	*P* value
Male sex[n(%)]	7(77.78)	13(1.25)	33(80.49)	12(85.71)	0.966
Age(years)	56.89 ± 9.29	51.19 ± 10.62	52.78 ± 13.27	47.50 ± 11.66	0.312
Diabetes duration(years)	11.67 ± 6.02	8.76 ± 6.55	9.43 ± 6.27	13.77 ± 7.36	0.423
SBP(mmHg)	146.67 ± 18.72	147.5 ± 26.13	145.49 ± 28.44	146.54 ± 18.25	0.995
DBP(mmHg)	86.11 ± 15.00	91.88 ± 20.19	82.78 ± 15.10	88.69 ± 10.33	0.489
eGFR (mL/min/1.73 m2)	91.12 ± 25.62	76.35 ± 32.07	63.17 ± 28.48	27.68 ± 12.29	<0.001
24 h urine protein(g/d)	1.11 ± 0.85	2.86 ± 2.93	5.05 ± 4.09	5.81 ± 3.55	<0.001
Hb(g/L)	129.00 ± 17.57	119.00 ± 22.81	105.07 ± 21.13	100.50 ± 21.11	0.003
HbA1C(%)	6.89 ± 2.33	7.14 ± 1.39	8.04 ± 1.90	8.65 ± 2.68	0.147
FBG(mmol/L)	7.14 ± 3.10	7.93 ± 5.33	8.15 ± 4.16	9.40 ± 9.94	0.877
Cr (umol/L)	81.27 ± 16.47	123.11 ± 101.15	130.63 ± 69.96	259.39 ± 93.33	<0.001
BUN(mmol/L)	6.67 ± 2.28	8.37 ± 2.63	9.09 ± 4.12	16.24 ± 6.60	<0.001
Uric acid(umol/L)	332.39 ± 56.17	384.28 ± 109.95	391.62 ± 105.76	424.71 ± 103.75	0.219
Total protein(g/L)	66.10 ± 6.98	56.98 ± 10.00	55.87 ± 7.32	57.69 ± 7.98	0.010
Albumin(g/L)	37.68 ± 4.04	31.97 ± 7.73	30.42 ± 5.76	32.84 ± 7.12	0.022
TC(mmol/L)	1.57 ± 0.66	1.44 ± 1.81	2.05 ± 1.94	2.76 ± 1.92	0.001
TG(mmol/L)	4.39 ± 0.59	5.21 ± 1.91	5.13 ± 1.58	5.59 ± 1.75	0.296
HDL(mmol/L)	1.14 ± 0.21	1.17 ± 0.35	1.09 ± 0.34	1.07 ± 0.41	0.653
LDL(mmol/L)	2.92 ± 0.40	3.58 ± 1.45	3.48 ± 1.31	3.86 ± 1.22	0.29
Cystatin C(mg/L)	1.59 ± 0.31	1.37 ± 0.66	2.09 ± 1.06	2.82 ± 0.66	0.008
PTH(pg/mL)	33.91 ± 21.46	72.07 ± 58.16	44.69 ± 30.19	112.01 ± 98.96	0.068
25(OH) vitamin D(nmol/L)	47.64 ± 24.81	32.55 ± 23.11	21.91 ± 11.94	21.22 ± 10.94	0.01
K(mmol/L)	4.10 ± 0.48	4.00 ± 0.63	4.00 ± 0.47	4.16 ± 0.75	0.809
Ca(mmol/L)	2.25 ± 0.07	2.13 ± 0.17	2.08 ± 0.13	2.18 ± 0.17	0.011
P(mmol/L)	1.24 ± 0.21	1.26 ± 0.30	1.24 ± 0.17	1.35 ± 0.25	0.595
Na(mmol/L)	139.69 ± 2.32	140.61 ± 2.34	141.14 ± 2.23	139.31 ± 3.33	0.137
Cl(mmol/L)	105.04 ± 1.97	106.63 ± 4.43	108.16 ± 3.09	106.13 ± 5.06	0.049
NS[n(%)]	0(0)	2(12.50)	14(34.15)	5(35.71)	0.074
Urine pH	5.62 ± 0.56	5.79 ± 0.46	6.01 ± 0.61	5.98 ± 0.66	0.299
Urine bicarbonate(mmol/L)	24.22 ± 1.48	24.31 ± 1.20	25.12 ± 2.19	26.07 ± 2.67	0.092
Urine titratable acid(mmol/L)	20.11 ± 12.55	17.19 ± 7.30	13.68 ± 9.83	10.00 ± 5.45	0.014
Urine ammonium(mmol/L)	26.22 ± 13.67	24.88 ± 14.86	24.24 ± 18.48	16.36 ± 9.88	0.067

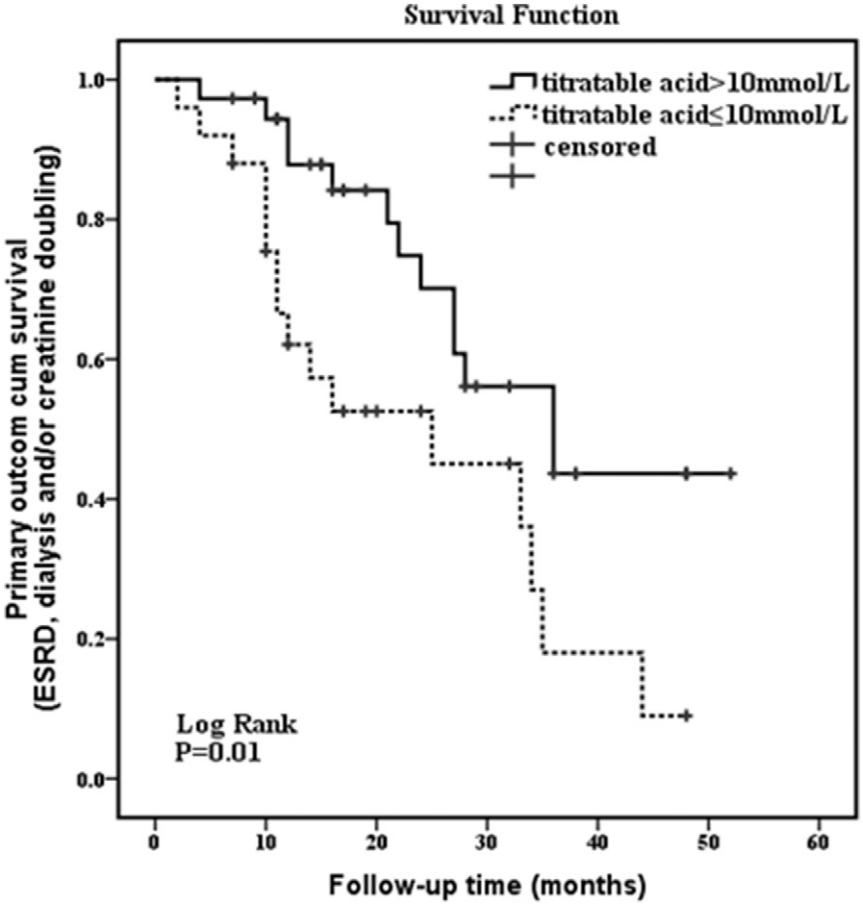

Fig. 2. Kaplan-Meier curves of the incidence of the renal outcomes according to urine titratable acid in 80 DKD patients.

Table 5
Univariate and multivariate Cox proportional hazard models at renal outcomes.

Variables	Univariable		Multivariable[a]	
	HR(95%CI)	p value	HR(95%CI)	p value
Cr	1.946(0.984–3.847)	0.056		
eGFR	1.641(1.136–2.370)	0.008		
24 h urine protein	2.991(1.347–6.642)	0.007	3.447(1.186–10.019)	0.023
Urine titratable acid	2.516(1.202–5.265)	0.014	2.306(1.025–5.187)	0.043
Urine bicarbonate	1.012(0.133–7.682)	0.990		
Urine ammonium	1.429(0.671–3.043)	0.355		

[a] Multivariable: adjusted for sex, age, blood pressure and diabetes duration. CI, confidence interval. HR, hazard ratio.

disorder group than the normal group. These findings are consistent with previous proposals that proteinuria occurs when the high levels of albumin filtered by glomeruli exceed the tubular reabsorption capacity[6]. Another possible explanation could be that glomerular proteinuria generates tubular damage and accelerates kidney disease progression[26].

The glomerular filtration rate is used to assess kidney function. Increased GFR, also called hyperfiltration, plays an important role in the initiation of kidney damage. Without therapeutic interventions, GFR falls progressively in parallel with a further rise in proteinuria which may lead to end-stage renal failure[27]. Fu *at al* found neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 excretions were increased in urine and were positively correlated with GFR, indicating that glomerular hyperfiltration is correlated with tubular dysfunction in the early stage of DKD[27]. In the current study, the level of baseline eGFR in the titratable acid disorder group (or in the ammonium secretion disorder group) was significantly lower than the normal group. The reduction of the baseline eGFR was associated with a disorder in bicarbonate reabsorption and the disorder in titratable acid or ammonium secretion. Those results demonstrated the GFR was still associated with tubular dysfunction in the progression of DKD. Moreover, the glomerular pathological classification of DKD suggested that the severity of glomerular lesions was correlated with titratable acid disorder. The previous study showed that the expression of a tubular oxidative injury marker p-p66Shc was different in pathological classification IIa, II b and III of DN patients, respectively[28]. Thus, significant correlations were observed between glomerular lesions and renal tubular damage, which is consistent with the viewpoint that tubulointerstitial changes are regularly associated with the progressive reduction of the GFR[29].

Baseline proteinuria and eGFR are established risk factors for the progression of DKD[30]. Recent studies focus on tubular damage markers for predicting the outcomes of DKD. Kim SS et al. found that urinary cystatin C is a strong predictor of renal impairment in patients with type 2 diabetic, which is independent of serum cystatin C[24]. Titan SM et al. suggested that FGF-23 is a biomarker for DKD progression in patients with microalbuminuria[31]. Our result showed that the DKD progression was significantly associated with the titratable acid disorder. The previous study demonstrated that lower urinary ammonia was associated with a higher risk of CKD progression[32]. Also, higher net acid excretion was associated with a lower risk of the progression to ESRD[33]. These studies supported our point that the risk of CKD progression was related to low acid excretion in diabetes, and tubular damage might play a significant role in renal impairment. Although we found that the most common urinary acidification dysfunction in our study was the disorder of ammonium secretion, a relationship between urinary ammonia excretion and the risk of DKD progression was not observed. In fact, renal ammonia excretion is regulated by a wide variety of conditions[34]. The mechanisms that caused abnormal ammonia metabolism, transport, and excretion might be different in different types of RTA[35]. Hence, lower ammonia excretion is complex and hard to accurately reflect the renal tubular injury and renal progression in diabetic kidney disease.

There are several limitations to this study. First, this is a study with a small sample size and short follow-up time. Second, this study included patients with advanced proteinuria. The findings may be different in patients with normoalbuminuria. Third, this study focused on the relationship between tubular injury and baseline eGFR. The relationship of tubular biomarkers with changes in GFR overtime was not observed. Finally, the titratable acid in the urine is mainly the monovalent organic phosphate. Loss of phosphorus homeostasis due to excretion failure in CKD patients might result in hyperphosphatemia[36]. In this study, there were no differences in serum phosphorus between groups with or without urinary acidification dysfunction. However, the excretion of phosphate was not examined.

5. Conclusion

In conclusion, we have demonstrated that the dysfunction of titratable acid secretion was an independent risk factor of DKD progression. Thus, titratable acid secretion may serve as a novel and promising biomarker to predict DKD progression.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Disclosure statement

The authors have no conflicts of interest to disclose.

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