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Increased urinary miR-196a level predicts the progression of renal injury in patients with diabetic nephropathy

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ABSTRACT

Background. Recent data suggest that miR-196a is predominantly expressed in the kidney and plays an inhibitory role in the progress of renal interstitial fibrosis (IF). However, the predictive value of miR-196a in diabetic nephropathy (DN) remains unknown. We validated the role of urinary miR-196a in the progression of renal injury in a cohort of patients with type 2 diabetes mellitus.

Methods. Our study included 209 patients with biopsy-proven DN. The mean follow-up time was 54.03 ± 32.94 months. Histological lesions were assessed using the pathological classification established by the Renal Pathology Society. Percentages of IF and tubular atrophy were assessed using the Aperio ScanScope system. We measured the correlation of urinary miR-196a with clinical and pathological parameters using the Spearman's correlation test. The influence of urinary miR-196a on renal outcomes was assessed using Cox regression analysis.

Results. Urinary miR-196a levels correlated positively with proteinuria ($\rho = 0.385$, $P < 0.001$), duration of diabetes mellitus ($\rho = 0.255$, $P < 0.001$) and systolic blood pressure ($\rho = 0.267$, $P < 0.001$). The baseline estimated glomerular filtration rate (eGFR) and hemoglobin level showed a negative correlation with urinary miR-196a ($\rho = -0.247$, $P < 0.001$ and $\rho = -0.236$, $P = 0.001$, respectively). Pathologically, urinary miR-196a levels correlated with glomerular sclerosis and IF in patients with DN. Urinary miR-196a was significantly associated with progression to end-stage renal disease [hazard ratio (HR) 2.03, $P < 0.001$]

and a 40% reduction of baseline eGFR (HR 1.75, P = 0.001), independent of age, gender, body mass index, mean arterial pressure and hemoglobinA1c level. However, urinary miR-196a did not improve predictive power to proteinuria and eGFR in DN patients.

Conclusions. Increased urinary miR-196a was significantly associated with the progression of renal injury and might be a noninvasive prognostic marker of renal fibrosis in DN patients.

Keywords: diabetes nephropathy, miR-196a, renal fibrosis

INTRODUCTION

Diabetic nephropathy (DN) is a progressive disease associated with an increased risk of mortality and cardiovascular events. In the past two decades, rates of diabetes-related cardiovascular events have declined substantially, but the overall prevalence of diabetic kidney disease has not changed significantly [1, 2]. In spite of aggressive multipronged management, many patients still rapidly progress to end-stage renal disease (ESRD) [3]. Several clinical factors associated with long-term outcome in patients with DN have been identified previously, such as hyperglycemia, hypertension and albuminuria [4, 5]. Recent studies suggest that pathological classification also has a significant impact on renal outcomes in patients with DN [6–9]. Our previous study demonstrated that the severity of glomerular and interstitial lesions was clearly associated with progression to ESRD and the doubling of serum creatinine, independent of clinical features [6].

The characteristic histological changes in DN are the accumulation of extracellular matrix materials, resulting in glomerular basement thickening, mesangial expansion, glomerular sclerosis and interstitial fibrosis (IF) [10]. These changes usually lead to an irreversible decline in renal function, and patients will ultimately require renal replacement therapy. Transforming growth factorβ (TGF-β) is reported to be the key fibrotic factor in DN both *in vitro* and *in vivo* [11–14]. TGF-β is involved in the synthesis of extracellular matrix proteins through activation of its downstream Smad and non-Smad signaling pathways, as well as through the hypertrophy, proliferation and apoptosis of renal cells [14]. An improved understanding of the regulation of TGF-β signaling may provide novel strategies for prevention of renal fibrosis.

Monitoring the progression of renal fibrosis with a noninvasive method has been another particularly challenging task. In the past few years, microRNAs (miRNAs) have garnered great interest and have been shown to be implicated in various human diseases, including DN [15–17]. Our previous study demonstrated that miR-196a was selectively expressed in the kidney and played an inhibitory role in the progression of renal fibrosis through downregulation of TGF-β receptor II (TGFβR2) [18]. Urinary miR-196a levels increased in patients with active focal segmental glomerular sclerosis (FSGS), DN and membranous nephropathy [19]. These findings indicated a potential application of urinary miR-196a as a noninvasive biomarker for renal fibrosis. In this study we aimed to evaluate the correlation of urinary miR-196a with clinical and pathological parameters, as well as renal outcomes, in a biopsy-proven DN cohort.

MATERIALS AND METHODS

Study design and population

We performed a retrospective study in a cohort of DN patients recruited from the Nanjing Diabetic Nephropathy Registry. The protocol has been described previously [6]. Briefly, patients with type 2 diabetes mellitus (T2DM) who had biopsy-proven DN, a biopsy specimen comprising ≥10 total glomeruli and a follow-up time of >12 months were eligible. Those who had comorbidities of nondiabetic renal diseases or acute kidney injury at the time of renal biopsy were excluded. DN was defined according to the following criteria: (i) a previous history of type 2 diabetes, usually concomitant with diabetic retinopathy, neuropathy and atherosclerotic complications; (ii) the presence of persistent albuminuria (≥30 mg/24 h) or decreased renal function [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2]; (iii) biopsy-proven kidney disease caused by diabetes and (iv) an exclusion of nondiabetic renal disease. After obtaining informed consent, first morning urine was collected before renal biopsy. Of the 396 patients enrolled from 2003 to 2011, a urine sample was available for 209 patients.

Sample preparation and quantification of miR-196a

Briefly, first morning urine specimens collected at room temperature were centrifuged at 1500 *g* for 10 min at 4°C. The supernatant from each sample was aliquoted into RNase-free tubes and stored at −80°C until testing. All samples were processed within 4 h after collection. Urine samples were thawed on ice and 300 μL of urine were used for total RNA extraction using TRIzol LS (Invitrogen, Waltham, MA, USA; catalog no. 10296028) according to the manufacturer's instructions.

We quantified urinary miR-196a with TaqMan probe–based quantitative reverse transcription polymerase chain reaction (qRT-PCR), as previously described (7900HT Sequence Detection System, Applied Biosystems, Waltham, MA, USA) [19, 20]. Since there is no current consensus on a housekeeping gene that can be used for normalization in qRT-PCR analysis of urinary miRNAs of interest, levels of urinary miR-196a were directly normalized to sample volume [21, 22]. The unit of urinary miR-196a level is femtomoles per liter. The detailed method is described in the Supplementary data (Methods).

Clinical and pathological information

Renal biopsies were performed in these patients between 2003 and 2010. Follow-up data were updated until August 2017. The baseline clinical characteristics were collected within 1 month of renal biopsy. Subsequent follow-up visits were performed two to four times per year based on the patient's individual condition. Proteinuria and biochemical indices were measured by routine laboratory procedures. Use of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) was defined as a previous medication of ACEI or ARB for >3 months at the time of biopsy. eGFR was estimated using the 2009 Chronic Kidney

Disease Epidemiology Collaboration creatinine equation [23]. The primary endpoint was renal survival, defined as the progression to ESRD (eGFR <15 mL/min/1.73 m^2 or the initiation of chronic renal replacement therapy). The secondary endpoint was a 40% reduction of baseline eGFR. Serum creatinine and eGFR for each visit were recorded to confirm whether the patient had developed to the endpoint. Patients who did not reach the endpoint were recorded using the information from their last follow-up visit. Unfortunately, information on death was not available in our registration system.

All of the biopsy specimens were categorized based on the pathological classification established by the Renal Pathology Society [24] and were scored by a single pathologist who was blinded to the clinical findings. In addition, we assessed the percentages of IF and tubular atrophy (TA) using the Aperio ScanScope system (LMD7000, Leica, Wetzlar, Germany). Sections were stained with Masson trichrome and periodic acid methenamine silver for light microscopy. We determined the area of IF and TA on digitized images using the Aperio Imagescope software (version 12.3). The percentages of fibrotic (seen as light blue in Masson trichrome images) and tubular atrophic area (from silver stain images) relative to the total area of the image field were calculated.

Statistical analysis

The data were analyzed using SPSS (version 18, SPSS, Chicago, IL, USA) or R (version 3.2.1, R Project for Statistical Computing, Vienna, Austria) software. Data are presented as the mean ± standard deviation for normally distributed data or as the median and interquartile range (IQR) for nonnormally distributed data. Nominal data are reported as percentages of the total number of patients [% (*n*)]. The means were compared using one-way analysis of variance. For categorical variables, the differences were analyzed using the chi-square test.

Since the urine miR-196 levels were obviously skewed, they were log-transformed. We used the Spearman's correlation test to determine the correlation of urinary miR-196a levels with baseline clinical and pathological parameters. Cumulative renal survival was estimated by the Kaplan–Meier method for each urinary miR-196a quartile and renal survival rates were compared among these stratified cohorts using the log-rank test. The Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the primary and secondary endpoints. Logistic regression and area under the curve (AUC) values from the receiver operating characteristic (ROC) curves were performed to assess the classification of different parameters. P-values <0.05 were considered statistically significant.

RESULTS

Baseline clinical characteristics of patients according to the level of urinary miR-196a

A total of 209 patients with biopsy-proven DN were enrolled in this study, including 26 patients with microalbuminuria (30–300 mg/day), 85 patients with macroalbuminuria (>300 mg/day) and normal eGFR and 98 patients with decreased eGFR (<60 mL/min/1.73 m^2). The mean baseline eGFR was 69.09 ± 34.49 mL/min/1.73 m^2. The mean urinary protein excretion rate was 2.59 ± 2.50 g/24 h. Of the 98 patients with decreased eGFR, there were 8 cases with urinary protein excretion <0.5 g/day, 4 cases between 0.5 and 1.0 g/day, 39 cases between 1.0 and 3.5 g/day and 47 cases ≥3.5g/day. The mean eGFR at baseline for each category was 44.76 ± 14.10, 35.57 ± 7.30, 35.73 ± 11.62 and 39.70 ± 11.35. The median follow-up time for all patients was 48 months (IQR 21.5–68). At the time of renal biopsy, the mean age was 50.08 ± 8.66 years. The median duration of known diabetes mellitus was 96 months (IQR 41.5–168). During a mean follow-up of 54.03 ± 32.94 months, ESRD had developed in 90 patients (43.1%). A 40% reduction of baseline eGFR was observed in 123 patients (58.9%). Survival curves of the primary endpoints according to proteinuria and baseline eGFR are shown in the Supplementary data, Figure S1.

Clinical variables for the patients according to the urinary miR-196a quartile distributions are listed in Table 1. As the level of urinary miR-196a increased, the duration of diabetes mellitus, blood pressure (especially systolic blood pressure) and urinary protein excretion rate increased, whereas eGFR and hemoglobin levels decreased. The baseline glycosylated hemoglobin level was generally similar in patients in different quartiles of urinary miR-196.

Urinary miR-196a correlates with clinical parameters

Several clinical parameters were identified to be associated with urinary miR-196a levels at the time of renal biopsy (Table 2). First, high urinary miR-196a levels correlated significantly with overt proteinuria ($\rho = 0.385$, $P < 0.001$), hypoalbuminemia ($\rho = -0.446$, $P < 0.001$) and hypercholesterolemia ($\rho = 0.304$, $P < 0.001$). Second, urinary miR-196a levels correlated negatively with declining renal function ($\rho = -0.247$, $P < 0.001$) and subsequent anemia ($\rho = -0.236$, $P = 0.001$). Third, the duration of diabetes mellitus and systolic blood pressure also showed a positive correlation with urinary miR-196a levels ($\rho = 0.255$, $P < 0.001$ and $\rho = 0.267$, $P < 0.001$, respectively). However, urinary miR-196a levels did not correlate with age, gender, BMI, triglyceride levels, hemoglobin A1c (HbA1c) or therapy of ACEI/ARB ($P > 0.05$).

Urinary miR-196a correlates with pathological parameters

We further investigated the correlation of urinary miR-196a levels with pathological parameters. The urinary miR-196a levels of patients with different pathological classifications of glomerular and interstitial lesions as defined by the Renal Pathology Society [24] are shown in Figure 1a, c and e. Urinary miR-196a levels correlated significantly with the percentages of glomerular sclerosis ($\rho = 0.198$, $P = 0.004$; Figure 1b) and segmental sclerosis ($\rho = 0.137$, $P = 0.048$). Furthermore, urinary miR-196a levels also correlated positively with the percentages of IF ($\rho = 0.220$, $P = 0.002$; Figure 1d) and TA ($\rho = 0.290$, $P < 0.001$; Figure 1f).

Table 1. Baseline characteristics of patients with DN according to quartiles of urinary miR-196a

Characteristics	Total ($N = 209$)	DN patients according to quartiles of urinary miR-196a				P-value
		Q1 ($n = 52$)	Q2 ($n = 53$)	Q3 ($n = 52$)	Q4 ($n = 52$)	
Age (years)	50.07±8.66	49.88±8.28	47.98±8.74	50.69±9.33	51.77±8.04	0.218
Male, % (n)	64.6 (135)	69.2 (36)	66.0 (35)	75.0 (39)	48.1 (25)	0.027
Duration of diabetes (months)	105±76	91±77	84±68	109±78	134±73	0.004
Body mass index (kg/m^2)	25.11±3.44	25.00±2.73	25.34±3.79	25.22±3.68	24.91±3.53	0.920
Systolic blood pressure (mmHg)	141±19	135±16	138±21	145±18	147±18	0.001
Diastolic blood pressure (mmHg)	84±10	83±9	83±11	85±9	87±10	0.108
Mean arterial pressure (mmHg)	103±11	100±10	101±13	105±11	107±11	0.007
Concentration of urinary miR-196a (fmol/L), median (IQR)	136 (69–296)	48 (35–59)	94 (78–113)	218 (174–249)	507 (352–1141)	<0.001
Urinary miR-196a (log10)	2.19±0.48	1.65±0.14	1.97±0.09	2.32±0.10	2.83±0.35	<0.001
24-h proteinuria (g/day)	2.59±2.50	1.62±1.89	2.07±2.40	2.98±2.50	3.69±2.69	<0.001
Serum creatinine (mg/dL)	1.38±0.73	1.22±0.68	1.31±0.74	1.49±0.79	1.50±0.67	0.134
eGFR (mL/min/1.73 m^2), median (IQR)	63.59 (39.05–100.42)	76.68 (52.65–109.61)	68.13 (40.92–110.75)	56.70 (37.79–83.57)	47.86 (31.13–76.77)	0.007
Serum albumin (g/L)	37.42±7.41	41.12±6.19	39.28±6.58	36.20±7.08	33.04±7.27	<0.001
Cholesterol (mmol/L)	5.18±1.81	4.65±1.15	4.84±1.86	5.20±1.61	6.05±2.17	<0.001
Triglycerides (mmol/L)	1.94±1.04	1.84±1.15	1.92±1.08	1.92±0.89	2.07±1.04	0.746
Fasting blood sugar (mmol/L)	6.41±2.22	6.93±2.44	6.87±2.25	5.55±1.98	6.28±1.94	0.004
HbA1c (mmol/mol) (%)	41±14 (6.55±2.30)	43±12 (6.90±1.91)	41±14 (6.60±2.33)	38±15 (6.07±2.39)	41±14 (6.63±2.51)	0.320
Hemoglobin (g/L)	117±23	122±21	119±25	117±22	108±20	0.013
Use of ACEI or ARB, % (n)	52.2 (109)	59.6 (31)	45.3 (24)	50.0 (26)	53.8 (28)	0.509
Progressed to ESRD, % (n)	43.1 (90)	25.0 (13)	39.6 (21)	46.2 (24)	61.5 (32)	0.002
40% decline in eGFR, % (n)	58.9 (123)	46.2 (24)	52.8 (28)	63.5 (33)	73.1 (38)	0.029

Values are presented as mean ± standard deviation unless stated otherwise. Q1–Q4, quartiles 1–4.

Table 2. Correlations between urinary miR-196a and clinical parameters

Clinical parameters	ρ	P-value
Age (years)	0.121	0.082
Gender	0.129	0.063
Body mass index (kg/m^2)	−0.041	0.564
Duration of diabetes (m)	0.255	<0.001
Systolic blood pressure (mmHg)	0.267	<0.001
Diastolic blood pressure (mmHg)	0.146	0.034
24-h proteinuria (g/day)	0.385	<0.001
eGFR (mL/min/1.73 m^2)	−0.247	<0.001
Serum albumin (g/L)	−0.446	<0.001
Cholesterol (mmol/L)	0.304	<0.001
Triglycerides (mmol/L)	0.116	0.094
Fasting blood sugar (mmol/L)	−0.171	0.013
HbA1c (%)	−0.031	0.665
Hemoglobin (g/L)	−0.236	0.001
Use of ACEI or ARB	−0.017	0.804

Association between urinary miR-196a and renal outcomes

Survival curves for the primary and secondary endpoints according to quartiles of urinary miR-196a level are shown in Figure 2. The Kaplan–Meier survival analysis showed an overall 5-year renal survival rate of 56.1% in these patients. For patients in the lowest to the highest (fourth) quartile, the 5-year renal survival rates were 74.7, 58.9, 48.3 and 37.3%, respectively. The HRs for different quartiles with respect to renal outcomes are shown in Figure 3. Compared with patients in the first quartile of urinary miR-196a level, the HRs for renal survival in the third and fourth quartile were 2.62 (95% CI 1.32–5.18) and 3.59 (95% CI 1.87–6.87), respectively.

In univariate Cox regression, urinary miR-196a levels were significantly associated with renal survival (HR 2.03, $P \leq 0.001$) and a 40% reduction of baseline eGFR (HR 1.75, $P = 0.001$). In multivariate Cox regression analysis, when adjusted for age, gender, body mass index, mean arterial pressure and HbA1c, urinary miR-196a remained an independent risk factor for renal survival (HR 1.77, $P = 0.008$) and a 40% reduction of baseline eGFR (HR 1.67, $P = 0.007$). However, the correlation of urinary miR-196a levels with renal outcomes disappeared when adjusted by proteinuria (Table 3). And the addition of urinary miR-196a did not improve predictive power to the traditional parameters (including eGFR and proteinuria), when evaluated by comparing the AUC curves of corresponding models (Table 4).

DISCUSSION

In this study we evaluated the correlation of urinary miR-196a with clinical and pathological parameters in a cohort of DN patients. The results demonstrated that (i) urinary miR-196a levels correlated with a variety of clinical parameters in patients with DN, such as baseline blood pressure, proteinuria, eGFR and the duration of diabetes mellitus; (ii) pathologically, urinary miR-196a levels correlated with glomerular sclerosis and IF in patients with DN and (iii) urinary miR-196a was significantly associated with renal outcomes (progression to ESRD and a 40% reduction in baseline eGFR) in DN patients.

The strengths of our study include the long-term follow-up cohort of patients with T2DM, in which we could observe unmistakable renal endpoints (43.1% of patients progressed to ESRD). Furthermore, the availability of renal biopsy specimens enabled us to investigate the relationship of urinary miR-196a to renal fibrosis with a quantitative analysis of renal fibrosis in our patients. The limitations of our study include its

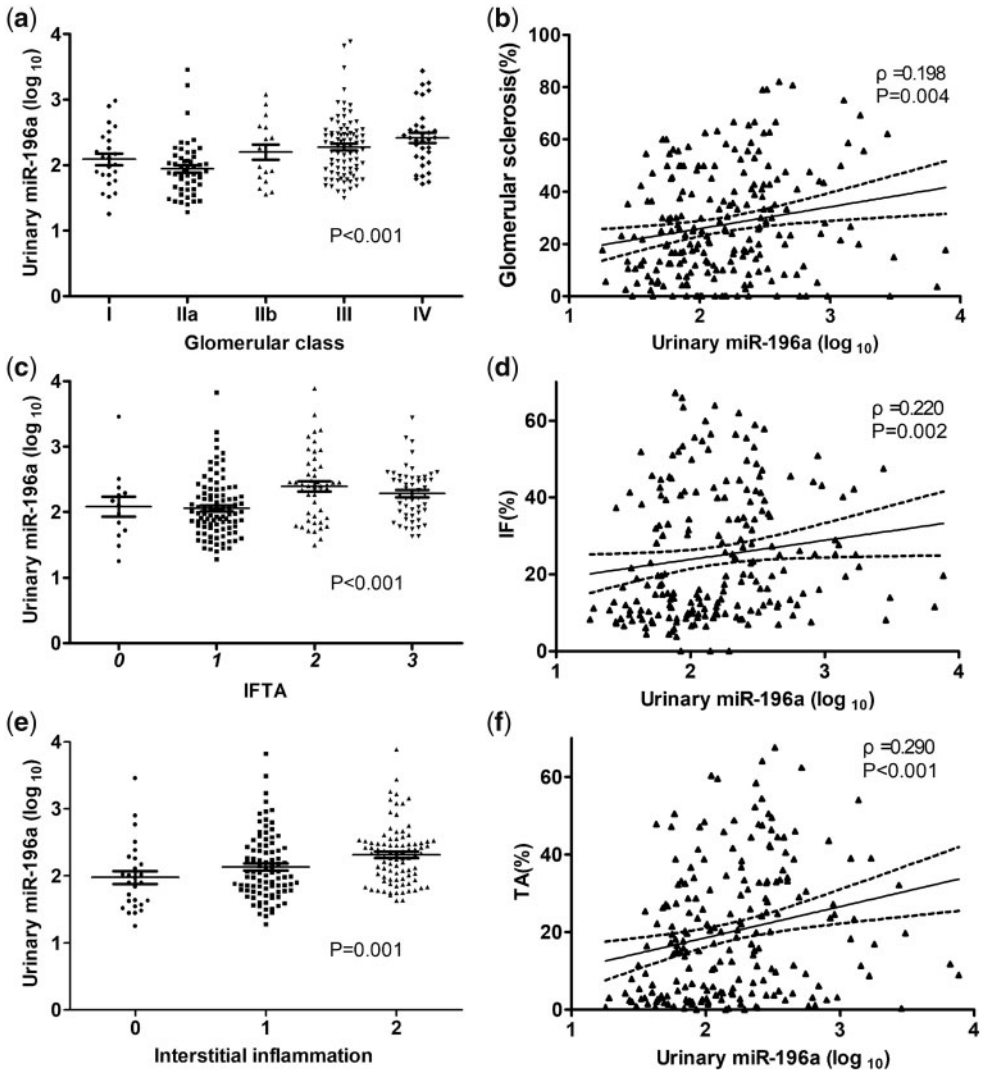

FIGURE 1: Urinary miR-196a in patients with various types of pathological damage. (**a**) Urinary miR-196a levels in patients with different classifications of glomerular changes. (**b**) Correlation of urinary miR-196a with glomerular sclerosis percentage. (**c**) Urinary miR-196a levels in patients with different IFTA scores. (**d**) Correlation of urinary miR-196a with IF percentage. (**e**) Urinary miR-196a levels in patients with different interstitial inflammation scores. (**f**) Correlation of urinary miR-196a with TA percentage.

retrospective observational design, which precludes definite conclusions from being made about a cause–effect relationship between proteinuria and urinary miR-196a level, and its lack of information about how many patients died during the follow-up. In addition, the concentration of urinary miR-196a was measured in spot urine samples and was not normalized to the urinary creatinine concentration.

Tissue-specific miRNAs play an essential role in maintaining organ homeostasis [25–29]. Studies have shown that miR-196a is kidney enriched in both mice and humans [18, 29]. Our previous work revealed that 74.37% of miR-196a is distributed in the mouse kidney [18]. In FSGS patients with nephrotic proteinuria, urinary miR-196a levels were increased significantly when compared with patients in remission [19]. It is noticed that the urinary, but not plasma, miR-196a level correlates with disease activity of FSGS [20]. The abundant levels of urinary miR-196a were mostly derived from kidney damage, not the overload in blood.

Previous work demonstrated that when compared with normal control, urinary miR-196a levels increased in patients with DN [19]. In this study, our data revealed that urinary miR-196a correlated with a variety of clinical determinants of DN progression (blood pressure, proteinuria, eGFR, course of diabetes, etc.), suggesting that miR-196a was involved in different pathways that lead to kidney damage in DN patients. The correlations between miR-196a levels and different parameters were statistically significant, but most of the coefficients were relatively weak. This might be partly limited by the number of patients enrolled in our study. In contrast, it demonstrated that miR-196a is not a specific marker for a single disease or pathway but is related to various mechanisms involved in renal injury. We did not find a positive correlation between urinary miR-196a and HbA1c. A potential limitation is the lack of a control group of T2DM patients without renal involvement. Further validation in cohorts of diabetic patients without renal

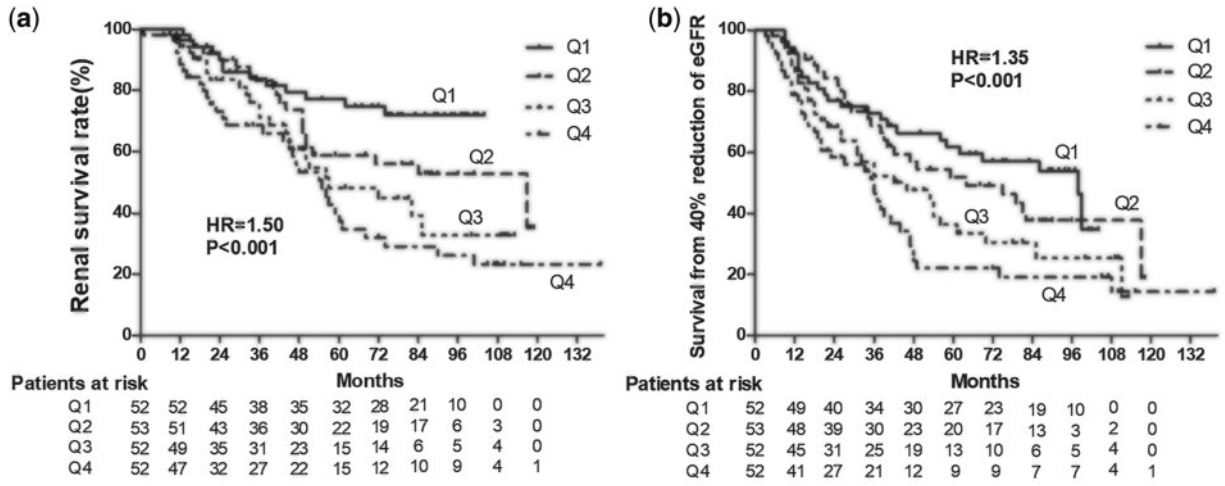

FIGURE 2: Kaplan–Meier curves for renal outcomes in patients with DN. (**a**) Renal survival rates of patients classified in different urinary miR-196a quartiles. (**b**) Rates of progression to a 40% reduction in baseline eGFR of patients classified in different urinary miR-196a quartiles.

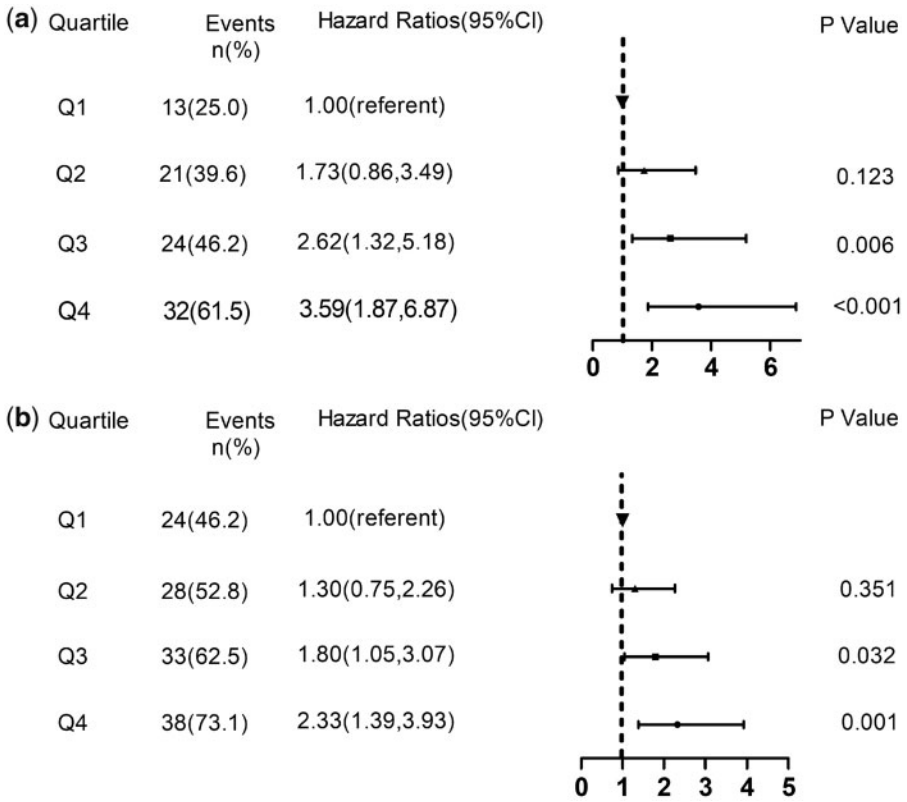

FIGURE 3: Associations between urinary miR-196a quartiles and renal outcomes in patients with DN. (**a**) Associations between urinary miR-196a quartiles and renal survival. (**b**) Associations between urinary miR-196a quartiles and progression to a 40% reduction in baseline eGFR.

injury will be required to investigate the influence of metabolic abnormality in miR-196a levels.

Renal fibrosis is the final histological result of various metabolic and hemodynamic abnormalities in DN. Previously, downregulation of miR-196a has been shown to increase type I collagen expression in scleroderma dermal fibroblasts and keloid fibroblasts [30–32]. Our previous work demonstrated a protective function of miR-196a in renal IF in the unilateral ureteral obstruction (UUO) mouse model of induced renal fibrosis [18]. Elevated expression of renal miR-196a significantly downregulated profibrotic proteins, whereas depletion of renal miR-196a substantially aggravated renal IF in the UUO model [18]. In a FSGS cohort, Zhang *et al.* [20] demonstrated that urinary miR-196a positively correlated with IF/TA, but intrarenal miR-196a displayed a negative correlation with IF/TA. The current research confirmed that urinary miR-196a correlated not

only with tubulointerstitial fibrosis but also with glomerular sclerosis. Levels of urinary miR-196a may reflect the severity of renal fibrosis.

We further investigated the correlation between urinary miR-196a and renal outcomes. The results showed that urinary miR-196a was significantly associated with progression to ESRD and a 40% reduction of baseline eGFR in patients with DN. Patients classified in the top quartiles of urinary miR-196a level tended to have a poor renal outcome. When adjusted for age, gender, body mass index, mean arterial pressure and HbA1c, urinary miR-196a remained an independent risk factor for renal survival. Thus urinary miR-196a may be a potential noninvasive marker for renal fibrosis with the ability to predict renal progression.

The underlying mechanism by which miR-196a modulates chronic kidney disease is not fully understood. Our previous work has shown that TGFβR2 mRNA is a common target of miR-196a in humans and mice [18]. Decreasing miR-196a expression in human HK2 cells strongly activated TGF-β/Smad signaling and cellular fibrosis, whereas increasing miR-196a levels in tubular epithelial cells inhibited TGF-β/Smad signaling [18]. It seemed that miR-196a played an inhibitory role in the progress of renal IF through downregulation of TGFβR2. However, the effect of miR-196a in intrinsic glomerular cells remains unknown. In this study we found a correlation between miR-196a levels and glomerular sclerosis. Both the glomerular and the tubular interstitial fractions contain abundant levels of miR-196a [18]. In FSGS, the expression of TGF-β1, TGFβR2 and phosphorylated Smad2/Smad3 in podocytes was significantly increased [33].

However, unlike what we found in an FSGS cohort [20], urinary miR-196a did not improve predictive power to proteinuria and eGFR in DN patients. In fact, the correlation of urinary miR-196a levels with renal outcomes in DN patients disappeared when adjusted by proteinuria. Overt proteinuria generally reflected podocyte injury in chronic kidney disease [34]. Proteinuria was reported to accelerate renal fibrosis through multiple pathways, including chemokine expression and complement activation [35, 36]. In contrast, DN is a disease with pathological diversity that is far more different from FSGS [37]. And podocyte injury is reversible to a great extent in FSGS. The association between miR-196a and podocyte injury needs to be further investigated in future research.

In conclusion, this study demonstrated that the urinary miR-196a level correlates with various clinical and pathological parameters. The urinary miR-196a level was significantly associated with renal outcomes when adjusted by clinical features. These results indicate that urinary miR-196a may be a noninvasive marker for the progression of renal fibrosis in patients with DN.

Table 3. Correlation of urinary miR-196a with renal outcome

Model	ESRD		40% reduction in baseline eGFR	
	HR (95%CI)	P-value	HR (95% CI)	P-value
Urinary miR-196a	2.03 (1.40–2.93)	<0.001	1.75 (1.26–2.45)	0.001
Model 1	1.92 (1.30–2.84)	0.001	1.74 (1.23–2.46)	0.002
Model 2	1.77 (1.16–2.71)	0.008	1.67 (1.15–2.43)	0.007
Model 3	1.58 (1.02–2.45)	0.040	1.41 (0.96–2.06)	0.080
Model 4	1.45 (0.98–2.16)	0.063	1.20 (0.84–1.72)	0.311

Model 1: adjusted by baseline age, gender and body mass index.
Model 2: adjusted by baseline age, gender, body mass index, mean arterial pressure and HbA1c.
Model 3: adjusted by baseline age, body mass index, mean arterial pressure, HbA1c and eGFR.
Model 4: adjusted by proteinuria.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

Y.A., C.Z. and Z.L contributed to the research idea and study design. Y.A., C.Z. and F.X. contributed to data acquisition. F.X. and C.Z. contributed to pathological analysis. Y.A. and W.L. contributed to data analysis. W.L. and L.X. contributed to statistical analysis. Z.L contributed to supervision or mentorship. Each author contributed important intellectual

Table 4. Evaluation of various prediction models

Model	ESRD			40% reduction of baseline eGFR		
	AUC	C-statistics (CI)	P-value	AUC	C-statistics (CI)	P-value
Urinary miR-196a (log10)	789	0.62 (0.56–0.68)		1083	0.61 (0.56–0.67)	
eGFR	692	0.82 (0.77–0.86)		1029	0.71 (0.66–0.75)	
Proteinuria	755	0.75 (0.70–0.81)		1036	0.75 (0.71–0.79)	
eGFR + proteinuria	677	0.83 (0.79–0.87)	–	1004	0.75 (0.71–0.80)	–
eGFR + proteinuria + miR-196a (log10)	679	0.83 (0.79–0.87)	0.93	1006	0.76 (0.71–0.80)	0.59
eGFR + proteinuria + miR-196a (quartile)	678	0.83 (0.79–0.87)	0.40	1004	0.76 (0.71–0.80)	0.22

content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

CONFLICT of INTEREST STATEMENT

None declared.

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