Urinary biomarkers of tubular injury to predict renal progression and end stage renal disease in type 2 diabetes mellitus with advanced nephropathy: A prospective cohort study

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ABSTRACT

Background: Novel potential tubular biomarkers in diabetic nephropathy could improve risk stratification and prediction. The study aimed to evaluate the association of tubular damage markers with rapid renal progression and incidence of end stage renal disease (ESRD) in type 2 diabetes (T2DM).
Methods: A prospective cohort study, involving a total of 257 patients with T2DM, was included. The baseline values of urine albumin, cystatin-C, angiotensinogen, kidney injury molecule-1 (KIM-1) and neutrophil-gelatinase associated lipocalin (NGAL) were measured. The composite outcomes included a rapid glomerular filtration rate (GFR) decline or incident of ESRD at 3-year follow-up.
Main findings: The composite outcomes were noted in 26.1%. Using univariate followed by multivariate COX proportional hazard regression analysis, the patients with highest quartiles of urine cystatin-C (HR 2.96, 95% CI, 1.38–6.35), urine angiotensinogen (HR 2.93, 95% CI, 1.40– 6.13) urine KIM-1 (HR 2.77, 95% CI, 1.27-6.05) and urine NGAL (HR 2.53, 95% CI, 1.11-5.76) were significantly associated with rapid renal progression when compared with the patients with the lowest quartiles of all tubular biomarkers.
Conclusions: Patients with T2DM with high levels of baseline urine tubular biomarkers (cystatin-C, angiotensinogen, KIM-1 and NGAL) had a greater incidence of ESRD and rapid GFR decline.

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1. Introduction

The global prevalence of type 2 diabetes mellitus (T2DM) in 2017 was 451 million prevalent cases and the prevalence will increase to 693 million cases by 2045, reflecting a remarkable increase in diabetes nephropathy.[1] According to the Thailand Renal Replacement Therapy Registry Report 2015, the most common etiology of dialysis prevalence is diabetes nephropathy (38.5%). Albuminuria is generally considered the earliest noninvasive marker for the development of nephropathy.[2] However, urine albumin is imprecise, does not directly measure renal tissue injury, and a large proportion of renal impairment occurs before the onset of albuminuria. Moreover, microalbuminuria has been shown to regress back to normoalbuminuria among patients with diabetic nephropathy.[3–5] Therefore, novel biomarkers in addition to albuminuria are needed to predict renal progression among patients with T2DM.

Pathology in diabetes nephropathy affects all the renal cellular elements including the glomerular endothelium, mesangial cells, podocytes and tubular epithelium.[6] Renal pathologic abnormalities are found among patient with T2DM before the onset of albuminuria.[7] Recently, renal tubulointerstitium has been increasingly reported to play an integral role in the pathogenesis of diabetes nephropathy and it predicts renal survival in both diabetic and nondiabetic glomerular disease.[8,9] With the development of diabetic nephropathy, tubulointerstitial damage is enhanced and the ability to reabsorb low molecular weight proteins is further reduced. Novel tubular biomarkers related to renal injury in diabetes nephropathy could improve risk stratification and prediction.

Urine cystatin-C, angiotensinogen, kidney injury molecule-1 (KIM-1) and neutrophil-gelatinase associated lipocalin (NGAL) are almost freely filtered by the renal glomeruli and almost entirely reabsorbed in the renal tubule like other low molecular weight proteins.[10–17] All renal tubular biomarkers constitute a sensitive index for tubular damage and may predict the progression of T2DM with nephropathy.[18] However, the clinical application of these biomarkers in predicting the progression of diabetic nephropathy remains uncertain.

To extend our previous short cohort study,[18] we have evaluated the role of novel urinary tubular biomarkers as predictors of decline in renal

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Data Availability Statement: All relevant data are within the paper.
Competing interests: The authors have declared that no competing interests exist.
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function and risk of ESRD in a longer prospective study of patients with T2DM and nephropathy.

2. Methods

2.1. Study population

Patients with T2DM presenting various degrees of renal impairment from the outpatient clinic, Department of Internal Medicine, Phramongkutklao Hospital from February 2014 to February 2015 were recruited in the study and followed up for a least 36 months. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Institute Review Board at the Royal Thai Army Medical Department. Informed consent forms were obtained from all the study participants. Inclusion criteria included age ≥18 years, estimated glomerular filtration rate (eGFR) ≥15 mL/min/1.73 m^2 and T2DM. Exclusion criteria included acute kidney injury episode, pregnancy, patient life expectancy <1 year and unwillingness to participate in the study.

2.2. Data collection

All patient history was carefully recorded by interview and confirmed by checking patient records and recording drug prescriptions. The demographic and clinical laboratory data were collected at baseline and the end of the study. Blood samples were taken in the morning before any food intake. Common biochemical parameters including urea, creatinine, hemoglobin A1C (HbA1C), serum lipids and electrolytes, albumin, hemoglobin and albuminuria were measured at baseline among all patients, according to standard methods in a routine clinical laboratory. Estimated GFR was assessed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[19]

2.3. Biomarkers measurement

Thirty milliliters of fresh urine were centrifuged at 4000 rpm for 10 min to remove particular impurities, then the supernatant stored frozen at −80 °C until assayed. All tubular biomarkers were tested by a commercially available employs quantitative sandwich ELISA technique according to the manufacturer's instructions. The urine tubular markers, angiotensinogen (R&D Systems China Co., Ltd.), cystatin-C (R&D Systems China Co., Ltd.), NGAL (R&D Systems Inc., USA & Canada) and KIM-1 (R&D Systems Inc., USA & Canada) were measured in urine samples at baseline. All specimens were diluted often to obtain concentration at the optimal density according to the ELISA kit instruction. Each test was performed in duplicate, according to product recommendations with coefficients of variation for variation <10%, for intra-assay and inter-assay variation. The enzymatic reactions were quantified in an automatic microplate photometer at 450–570 nm (Sunrise™ Absorbance Reader, TECAN Group Ltd., Switzerland). Urine NGAL and KIM-1 levels were expressed as nanograms per gram of creatinine, cystatin-C levels were expressed as micrograms per gram of creatinine while urine angiotensinogen levels were expressed as micrograms per gram of creatinine.

2.4. Assessment of outcomes

The primary composite renal outcomes were time to development of ESRD or sustained decline in eGFR of >5 mL/min/1.73 m^2 annually and were evaluated from the time of the patients' first urine sample and until February 2018.

2.5. Statistical analyses

Descriptive statistics are expressed as means ± standard deviation, median with interquartile range or percentage frequency, as appropriate. The t-test was used to compare continuous variables and chi-square test was used to compare categorical variables. Sensitivity, specificity, and area under the curve (AUC) were calculated as measures of diagnostic accuracy. Receiver operating characteristic (ROC) curves were used to calculate the AUC for urinary biomarkers and to find the best cut-off values of urine tubular biomarkers to identify the progression to renal endpoint. A perfect test has an area under the ROC curve of 1.0 and a faulty test has a value of ≤0.5. Kaplan-Meier curves were generated to assess renal survival among subjects with each cut-off point value of tubular biomarkers above and below the optimal ROC-derived cut-off levels. Cumulative incidences of renal progression were calculated using multivariate Cox proportional hazard regression analysis. A multivariable logistic regression analysis was performed to determine the predictors. A P-value <0.05 was considered significant (two-tailed). Data were analyzed using the commercially available SPSS 16.0 statistical software program (SPSS, Chicago, IL, US).

3. Results

3.1. Baseline characters of study population

Two hundred fifty-seven patients with T2DM were included in the study. The patients were followed for a median (range) of 40.8 (38.2–42.5) months. Successful follow-up until the end point was available in 97.2% of all patients. During follow-up to 2018, the cumulative incidence of rapid GFR decline was 37 patients (14.4%), ESRD was 30 patients (11.7%), and mortality rate was 7 patients (2.7%).

Baseline characteristics of the patients, when divided in groups according to the later development of renal endpoint are listed in Table 1. At baseline, mean age of patients was 66.2 ± 10.6 years, and more than one half were male (54.1%). Most study subjects had baseline albuminuria >30 mg/day (microalbuminuria 34.6% and macroalbuminuria 28.8%) and eGFR <60 mL/min/1.73 m^2 (56.8%). Regarding clinical and laboratory data, mean estimated GFR was 57 ± 26.3 mL/min/1.73 m^2, and HbA1c was 56.3 ± 11.6 mmol/moL; (7.3 ± 1.5%). The presence of diabetic complications including hyperlipidemia and anemia, was significantly more prevalent among patients with rapid GFR decline or development of ESRD. In addition, these patients had higher baseline urine albumin and intact-parathyroid hormone levels.

3.2. Urinary tubular biomarkers according to rapid renal progression

The effect of different statuses of renal progression on the levels of each biomarker was represented by box plots, as shown in Fig. 1. The median value of biomarkers including urinary cystatin-C [4.09 (1.24–9.27) vs. 2.72 (0.86–5.05) μg/g, P = 0.001], urinary angiotensinogen [4.67 (0.69–18.62) vs. 1.77 (0.25–5.92) μg/g, P = 0.001], urinary KIM-1 [90.1 (45.9–197.3) vs. 60.8 (27.5–112.3) ng/g, P = 0.007] and urinary NGAL [858.3 (559.3–1573.9) vs. 628.6 (378.9–1146.1) ng/g, P = 0.018] had significantly increased among patients with rapid renal progression or ESRD as shown in Fig. 1.

3.3. Biomarkers sensitivity and specificity

Sensitivity, specificity and AUC were calculated to assess the significance of the prognostic value for each biomarker in the rapid GFR decline or ESRD when compared with patients without rapid GFR decline or ESRD. ROC analysis showed an AUC for urinary cystatin-C, angiotensinogen, KIM-1, NGAL and albuminuria of 0.64 (95% CI, 0.56 to 0.72), 0.64 (95% CI, 0.55 to 0.72), 0.61 (95% CI, 0.53 to 0.69), 0.60 (95% CI, 0.52 to 0.68) and 0.73 (95% CI, 0.65 to 0.81), respectively as shown in Fig. 2(A). The best cut-off level for urine cystatin-C was 3.23 μg/g (sensitivity 61.2%, specificity 61.6%), urine angiotensinogen was 1.63 μg/g (sensitivity 68.7%, specificity 47.4%), urine KIM-1 was 70.47 ng/g (sensitivity 58.2%, specificity 55.3%) and urine NGAL was

Table 1

Baseline characteristics and laboratory data.

Parameters	All patients (N = 257)	Rapid GFR decline or ESRD (N = 67)	No rapid GFR decline and no ESRD (N = 190)
Age (year)*	66.2 ± 10.6	63.4 ± 10.7	67.2 ± 10.4
Male (%)	139 (54.1%)	33 (49.3%)	106 (55.8%)
Duration of DM (years)	10 (5, 17)	10 (2, 16)	10 (5, 20)
Follow up time (months)	40.8 (38.2, 42.5)	41.3 (37.9, 43.4)	40.6 (38.6, 42.3)
Comorbid disease			
CKD staging			
CKD I	39 (15.2%)	10 (14.9%)	29 (15.3%)
CKD II	72 (28%)	18 (26.9%)	54 (28.4%)
CKD III–IV	146 (56.8%)	39 (58.2%)	107 (56.3%)
Hypertension (%)	244 (94.9%)	63 (94%)	181 (95.3%)
Dyslipidemia (%)*	238 (92.6%)	58 (86.6%)	180 (94.7%)
Cardiovascular disease (%)	10 (3.9%)	4 (6%)	6 (3.2%)
Anemia (%)*	127 (49.6%)	42 (62.7%)	85 (45%)
Medications			
RAAS blocker (%)	163 (63.4%)	44 (65.7%)	119 (62.6%)
Insulin (%)	61 (23.7%)	18 (26.9%)	43 (22.6%)
ASA (%)	158 (61.5%)	39 (58.2%)	119 (62.6%)
Clinical parameter			
Systolic blood pressure (mmHg)	139.4 ± 18.6	141.5 ± 19.8	138.6 ± 18.2
Diastolic blood pressure (mmHg)	76.7 ± 11.6	78.2 ± 11.5	76.2 ± 11.6
Body mass index (kg/m²)	27.1 ± 4.9	27.9 ± 5.8	26.8 ± 4.6
Laboratory parameter			
Serum creatinine (mg/dL)	1.4 ± 0.7	1.6 ± 0.9	1.3 ± 0.5
GFR (mL/min/1.73 m²)	57.2 ± 26.3	54.4 ± 29.1	58.1 ± 25.3
UACR (mg/g)*	60.8 (10.7, 325.4)	395.8 (32.3, 1763.3)	42.9 (8.9, 166.7)
Fasting plasma glucose (mg/dL)	142.5 ± 58.7	138.7 ± 47.9	143.9 ± 62.1
Hemoglobin A1c (mmoL/moL)	56.2 ± 10.4	55.4 ± 12.3	56.3 ± 11.6
Hemoglobin (g/dL)*	12.2 ± 1.6	11.4 ± 1.8	12.5 ± 1.4
Serum phosphate (mg/dL)	3.4 ± 0.6	3.6 ± 0.9	3.4 ± 0.5
Intact-PTH (pg/mL)*	72 (54.3, 171.2)	157.8 (89.5, 256.6)	58.3 (43.3, 72)

DM: Diabetes Mellitus; CKD: Chronic Kidney Disease; RAAS blocker: Renin Angiotensin Aldosterone System; ASA: Aspirin; GFR: Glomerular Filtration Rate; UACR: Urine Albumin Creatinine Ratio; PTH: Parathyroid hormone.

* $P < 0.05$ vs. control group; note: values for categorical variables are given as number (percentage); values for continuous variables, as mean ± standard deviation or median [interquartile range].

614.14 ng/g (sensitivity 70.1%, specificity 48.4%). All tubular biomarkers demonstrated intermediate performance to predict rapid renal progression among patients with T2DM. However, the highest ROC integrated with urine albumin, angiotensinogen and NGAL was 0.75 (95% CI, 0.68 to 0.82) as shown in Fig. 2(B).

Kaplan-Meier survival curves among patients with urinary biomarkers above and below the optimal cut-off are presented in Fig. 3. Across all urine tubular biomarkers, the Kaplan–Meier curves for rapid GFR decline or development of ESRD showed a clear separation between patients stratified by high vs low baseline urine biomarker concentrations (urinary cystatin-C, angiotensinogen, KIM-1 and NGAL above 3.23 μg/g ($P < 0.001$, log-rank test), 1.63 μg/g ($P = 0.01$, log-rank test), 70.47 ng/g ($P = 0.026$, log-rank test), and 614.14 ng/g ($P = 0.002$, log-rank test), respectively).

Univariate analysis showed significantly increased risk for primary renal endpoint in the group of upper quartiles of all urine tubular biomarkers when compared with lower quartiles (Table 2). Multiple Cox regression analysis after adjusting potential risks for renal progression revealed all urine tubular biomarkers including urinary cystatin-C, angiotensinogen, KIM-1 and NGAL were still significantly associated with rapid renal progression at the end of the study. In a multivariate

logistic regression analysis, higher levels of urinary cystatin-C, angiotensinogen, KIM-1, and albuminuria were significantly associated with rapid renal progression (Table 3).

4. Discussion

In this study, we used four renal tubular injury biomarkers including urinary cystatin-C, angiotensinogen, KIM-1 and NGAL, in addition to albuminuria, to predict the composite outcome including GFR decline rate and ESRD among patients with T2DM and nephropathy. Our findings showed that urinary cystatin-C, angiotensinogen, KIM-1 and NGAL were significantly associated with rapid decline in renal function or development of ESRD and the performance of these biomarkers was similar to standard urinary albumin (AUC 0.6–0.7) among patients with T2DM and nephropathy.

Current research is focusing on a different strategy to enhance the sensitivity of biomarkers to predict renal progression or risk of ESRD among patients with T2DM. The importance of pathogenesis involving diabetic nephropathy, including the processes of genetic, environmental, inflammatory and biological factors with some of the promising new biomarkers in urine, are directly or indirectly related to these pathogenic mechanisms.[20] Different studies have underlined the crucial role played by renal tubule injury and the risk of kidney disease progression that closely correlates with the extent of tubulointerstitial fibrosis, regardless of etiology.[21] Novel biomarkers of the processes, that induce these tubulointerstitial changes, may ultimately prove to be predictors of disease progression and long term renal prognosis. Urinary cystatin-C, angiotensinogen, KIM-1 and NGAL are established early tubular biomarkers among patients of acute kidney injury and can be detected among patients before the development of glomerular damage or albuminuria.[22–25] All of these urine biomarkers are also considered to reflect declined GFR and the degree of tubulointerstitial fibrosis.[3] However, some studies have shown conflicting results.[25,26] In our study, we also confirmed in a longer cohort study that increased baseline levels of urinary tubular biomarkers were related to rapid renal progression or ESRD. The results of adjusted Cox proportional hazards modeling to predict rapid GFR decline also indicated that all tubular biomarkers had clinical value in predicting the GFR decline rate among patients with T2DM and nephropathy. However, this does not determine the mechanism or the role of these biomarkers.

Cystatin-C is easily filtered by the glomeruli and is re-absorbed and catabolized by the proximal tubule. Elevated urine cystatin-C has been recognized as a marker of renal tubular dysfunction.[27] Initially, in prospective observational studies, urine cystatin-C along with albuminuria was significantly associated with the annual decline in eGFR in type 2 diabetic nephropathy.[18,28] Our results also showed that urine cystatin-C was an independent predictor of renal progression among patients with T2DM and nephropathy in a long term study, which is consistent with the results of previous studies.

NGAL is expressed in the renal tubular epithelium, and a rise in urinary concentrations after acute renal injury.[16] The overexpression of NGAL detected in distal tubular cells in the setting of CKD[29] and level of urine NGAL are related to severity of nephropathy.[30] This may be due to the protective response of renal tubular NGAL in response to metabolic and hemodynamic stress. In observational follow-up, urine NGAL predicted albuminuria and progression of diabetic nephropathy[31] and our study also demonstrated that baseline levels of urine NGAL predicted rapid GFR decline among patients with T2DM and nephropathy, which is supported by other studies.[18,24]

KIM-1 increased expression on the membrane of proximal tubule cells with inflammation and fibrosis.[32] Related studies have shown that urine KIM-1 level was significantly associated with declined GFR among patients with type 1 diabetes[33] and patients with vasculitis or glomerulonephritis.[34] On the other hand, urine KIM-1 was not associated with declined renal function in other CKD populations and did not improve the clinical model predicting CKD progression.[35] Our
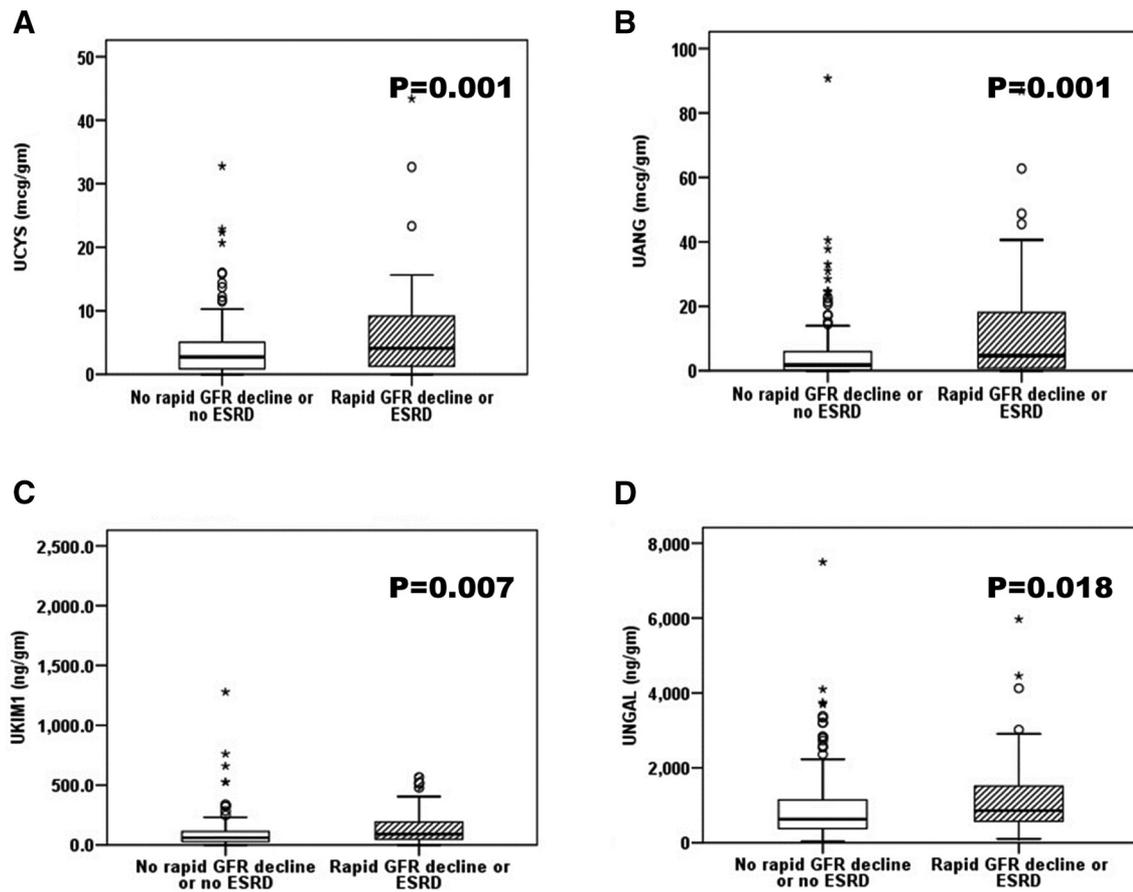
Fig. 1. Urinary levels of tubular biomarkers in rapid GFR decline or ESRD group and no rapid GFR decline or ESRD group. (A) Cystatin-C, (B) Angiotensinogen, (C) KIM-1, and (D) NGAL adjusted by urinary creatinine among T2DM patients classified in two groups: rapid renal progression or ESRD group and nonrapid renal progression or no ESRD group. Results are presented as median with interquartile range. UCYS: urine cystatin-C creatinine ratio; UANG: urine angiotensinogen creatinine ratio; UKIM-1: urine kidney injury molecule-1 creatinine ratio; UNGAL: urine neutrophil gelatinase associated lipocalin creatinine ratio.

results confirmed that urinary KIM-1 excretion was a predictor of tubulo-interstitial damage among patients with T2DM and nephropathy. The reasons for the heterogeneity of these results comprise potential states of acute chronic injury and differences in the underlying renal pathology.

Presence of high urinary angiotensinogen levels were detected in patients with increased renal angiotensin II and treatment with angiotensin receptor blocker reduced urinary angiotensinogen levels.[36] The potential of urinary angiotensinogen as a tool for evaluation of intrarenal angiotensin II activity is related to the degree of tubulointerstitial damage in diabetic nephropathy.[25] In diabetes without albuminuria, increased urine angiotensinogen level was also detected when compared with control subjects.[17] Our findings concerning urinary angiotensinogen excretion as a predictor of GFR
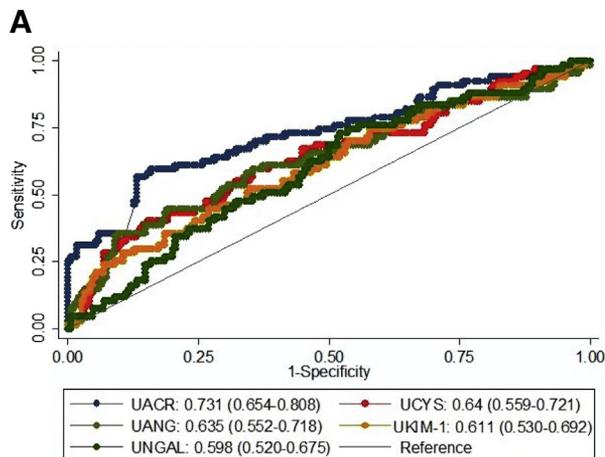
Biomarkers	AUC	95% CI	P value
UACR	0.731	0.65 – 0.81	<0.001
UACR+UCYS	0.744	0.67 – 0.82	<0.001
UACR+UNGAL	0.745	0.67 – 0.82	<0.001
UACR+UCYS+UNGAL	0.748	0.68 – 0.82	<0.001
UACR+UANG+UNGAL	0.751	0.68 - 0.82	<0.001
UACR+UCYS+UANG+UNGAL	0.745	0.67 - 0.82	<0.001

Fig. 2. (A) Graph ROC curves showing AUC of each tubular biomarker to predict rapid GFR decline or ESRD. (B) AUC of integrated tubular biomarkers with UACR to predict rapid GFR decline or ESRD. UCYS: urine cystatin-C creatinine ratio; UANG: urine angiotensinogen creatinine ratio; UKIM-1: urine kidney injury molecule-1 creatinine ratio; UNGAL: urine neutrophil gelatinase associated lipocalin creatinine ratio; UACR: urine albumin creatinine ratio.
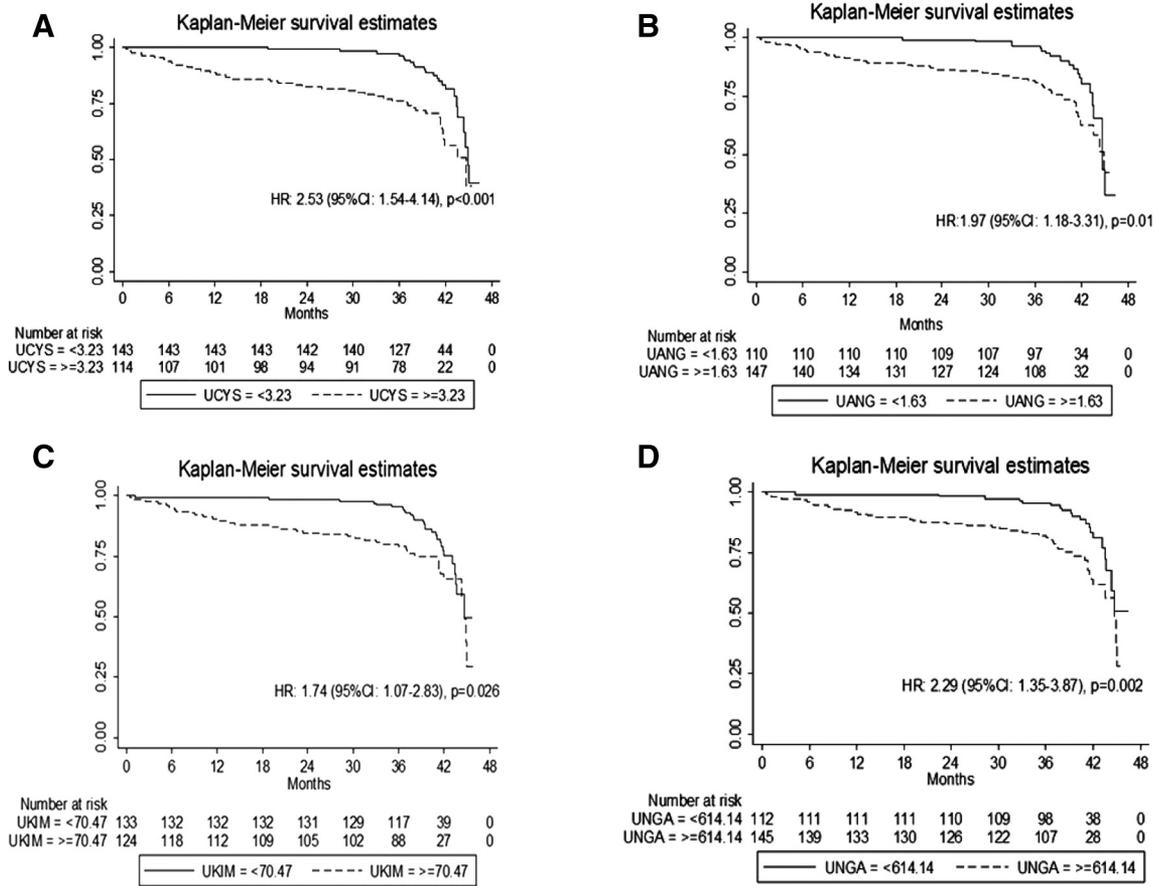
Fig. 3. Kaplan-Meier survival curves of renal endpoint among patients with UCYS, UANG, UKIM-1, UNGAL levels above and below the optimal ROC cut-off level of each tubular biomarker. (A) Patients with UCYS ≥3.23 μg/g (P < 0.001, log-rank test), (B) UANG ≥1.63 μg/g (P = 0.01, log-rank test), (C) UKIM-1 ≥ 70.47 ng/g (P = 0.026, log-rank test), (D) UNGAL ≥614.14 ng/g (P = 0.002, log-rank test) showed a significantly faster progression to endpoint.

decline in T2DM and nephropathy are in agreement with several other studies.[18,37]

Our study integrated novel tubular biomarkers at the same time and in a population with urine albumin, but it could not improve accuracy in predicting rapid renal progression in the setting of T2DM with significant albuminuria. The main reason could be that our study included 257 patients with diabetes with varying degrees of diabetic nephropathy and most had moderate to severe diabetic nephropathy (albuminuria >30 mg/day; eGFR <60 ml/min/1.73 m^2) at baseline of study. Therefore, a further study should be conducted among patients with T2DM without albuminuria to demonstrate the benefits of novel renal tubular biomarkers.

The results of the study are subject to some limitations. First, the study comprised a single center study and most of T2DM subjects included patients in advanced stages of diabetic nephropathy (up to 60% of patients were CKD stage III-IV) with unhomogenized patient characteristics. It might have affected the study outcomes. Second, the study employed a prospective observational cohort design. The lack of a control or intervention group for comparison might have limited the power of the study. Finally, urinary biomarkers might be a marker of filtration barrier damage rather than tubular damage in advanced CKD with high level of plasma biomarkers.[38] Further studies are needed to measure plasma and urine biomarkers levels at the same time. However, urinary tubular biomarkers have been shown to be a good predictor of renal

Table 2
Cox proportional hazards modeling to predict rapid GFR decline or ESRD.

Predictor variables	UCYS (HR [95%CI])	P-value	UANG (HR [95%CI])	P-value	UKIM-1 (HR [95%CI])	P-value	UNGAL (HR [95%CI])	P-value
Unadjusted								
First quartile	Reference		Reference		Reference		Reference	
Second quartile	1.05 (0.47, 2.33)	0.913	1.07 (0.48, 2.37)	0.864	1.78 (0.82, 3.86)	0.142	1.55 (0.71, 3.38)	0.27
Third quartile	1.4 (0.66, 3)	0.382	1.18 (0.53, 2.63)	0.694	1.6 (0.74, 3.46)	0.228	1.99 (0.94, 4.22)	0.073
Fourth quartile	3.86 (1.95, 7.66)	<0.001	3.93 (1.95, 7.88)	<0.001	3.41 (1.66, 7.01)	0.001	3.25 (1.58, 6.71)	0.001
Adjusted model with baseline of age, sex, body mass index, dyslipidemia, anemia and UACR								
First quartile	Reference		Reference		Reference		Reference	
Second quartile	1 (0.43, 2.3)	0.995	1.23 (0.55, 2.73)	0.615	1.98 (0.9, 4.32)	0.088	1.08 (0.47, 2.5)	0.86
Third quartile	1.2 (0.53, 2.72)	0.657	1.12 (0.47, 2.66)	0.792	1.04 (0.45, 2.41)	0.932	1.81 (0.83, 3.93)	0.133
Fourth quartile	2.96 (1.38, 6.35)	0.005	2.93 (1.4, 6.13)	0.004	2.77 (1.27, 6.05)	0.011	2.53 (1.11, 5.76)	0.027

UCYS: urine cystatin-C creatinine ratio; UANG: urine angiotensinogen creatinine ratio; UKIM-1: urine kidney injury molecule-1 creatinine ratio; UNGAL: urine neutrophil gelatinase associated lipocalin creatinine ratio; HR, hazard ratio; CI, confidence interval.

Table 3
Univariate and multivariate logistic regression analysis to predict rapid GFR decline or ESRD.

	Univariate analysis		Multivariate logistic regression analysis		Multivariate logistic regression analysis		Multivariate logistic regression analysis		Multivariate logistic regression analysis		Multivariate logistic regression analysis	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age	0.97 (0.94, 0.99)	0.012	0.98 (0.95, 1.01)	0.224	0.97 (0.94, 1)	0.028	0.96 (0.93, 0.99)	0.016	0.96 (0.93, 0.99)	0.006	0.96 (0.93, 0.99)	0.007
BMI	1.04 (0.99, 1.1)	0.138	1.01 (0.95, 1.08)	0.78	1.03 (0.97, 1.09)	0.371	1.02 (0.96, 1.08)	0.609	1.02 (0.96, 1.09)	0.443	1.03 (0.97, 1.09)	0.366
Dyslipidemia	0.36 (0.14, 0.92)	0.034*	1.03 (0.28, 3.78)	0.961	0.5 (0.18, 1.39)	0.182	0.54 (0.19, 1.54)	0.249	0.56 (0.2, 1.59)	0.278	0.45 (0.17, 1.22)	0.118
GFR	0.99 (0.98, 1.01)	0.321	1.01 (0.99, 1.02)	0.218	1 (0.98, 1.01)	0.527	1 (0.98, 1.01)	0.458	0.99 (0.98, 1.01)	0.273	0.99 (0.98, 1)	0.127
UACR	1.01 (1.01, 1.02)	<0.001*	1.01 (1.01, 1.02)	<0.001*								
UCYS	1.09 (1.03, 1.15)	0.001*			1.09 (1.02, 1.15)	0.006*						
UANG	1.04 (1.02, 1.07)	0.001*					1.04 (1.01, 1.07)	0.004*				
UKIM-1	1.01 (1.01, 1.02)	0.024*							1.01 (1.01, 1.02)	0.011*		
UNGAL	1 (0.99, 1)	0.099									1 (0.99, 1.01)	0.083

UCYS: urine cystatin-C creatinine ratio; UANG: urine angiotensinogen creatinine ratio; UKIM-1: urine kidney injury molecule-1 creatinine ratio; UNGAL: urine neutrophil gelatinase associated lipocalin creatinine ratio; HR, hazard ratio; CI, confidence interval.

injury prior to detectable changes in GFR. Moreover, the tubular biomarker molecule is freely filtered by the glomerulus and reabsorbed in the proximal tubules; its secretion in urine occurs after direct renal insults. According to experimental models, elevated NGAL and KIM-1 appeared to derive from impaired reabsorption in proximal tubules.[39,40] We suggested that urine biomarkers reflected damage to glomeruli, and tubules. On the other hand, this study draws its strength from assessing the prognostic values of various tubular biomarkers in different stages of diabetic nephropathy and our study employed a prospective cohort design in a long term follow-up period. Most notably, >90% were followed to 2018 or to the development of a renal end point.

5. Conclusion

The study supported that patients with T2DM and nephropathy with high levels of urine tubular biomarkers (cystatin-C, angiotensinogen, KIM-1 and NGAL) had a greater incidence of ESRD and rapid GFR decline at 3-year follow-up. These tubular biomarkers served as independent factors associated with renal progression among patients with T2DM and nephropathy. Further longitudinal prospective studies are needed to evaluate the predictive power of those markers to detect diabetic nephropathy in early stage diabetic nephropathy or T2DM without significant albuminuria.

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