Urine Biomarkers of Tubular Injury Predict Outcomes in Diabetic Nephropathy: A Prospective Cohort Study

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Abstract

Background: Currently available biomarkers, such as serum creatinine and albuminuria, exhibit low sensitivity in predicting renal progression. Novel biomarkers of tubular injury may aid in identifying patients with type 2 diabetes mellitus (T2DM) at high risk for renal progression. This study evaluates the potential of urine biomarkers of tubular damage in predicting renal progression and the composite outcome of renal progression and death in T2DM.

Methods: This prospective cohort study involved 257 patients with T2DM. Urine biomarkers of tubular injury were assessed at baseline. The outcomes examined were the composite renal outcome of end-stage kidney disease (ESKD), a 40% decline in eGFR, and death.

Results: Most patients were in chronic kidney disease (CKD) stages 3 and 4, with a median urine albumin/creatinine ratio of 60.8 mg/g. The median follow-up duration was 7 years. Baseline urine concentrations of cystatin-C, angiotensinogen, kidney injury molecule-1 (KIM-1), and neutrophil-gelatinase associated lipocalin (NGAL) were significantly higher among patients who reached the composite renal outcome. All tubular biomarkers demonstrated intermediate predictive performance for the composite renal outcome, with area under the curve (AUC) values ranging between 0.65 and 0.72, comparable to urine albumin/creatinine. Using the optimal cut-off value for each urine biomarker, higher levels were significantly associated with the composite renal outcome. However, when employing an adjusted Cox proportional hazards model for the composite renal endpoint across the quartiles of urine tubular biomarker levels, only the upper quartiles of urine cystatin-C and KIM-1 significantly predicted the composite renal endpoint.

Conclusion: Urine biomarkers of tubular injury effectively identified diabetic patients at elevated risk for CKD progression and death in T2DM patients

Keywords: urine biomarkers; CKD; kidney failure; ESRD; diabetes; DM; diabetic nephropathy; proteinuria

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ตัวบ่งชี้ทางชีวภาพในปัสสาวะอาจสามารถพยากรณ์ผลลัพธ์ในผู้ป่วยเบาหวานชนิดที่ 2: การศึกษาแบบไปข้างหน้า

อธิษฐ์ สุวรรณทศ, เนาวนิตย์ นาทา, อุปถัมภ์ ศุภสินธุ์, บัญชา สถิระพจน์

สาขาโรคไต แผนกโรคไต กองอายุรกรรม โรงพยาบาลพระมงกุฎเกล้า

บทคัดย่อ

บทนำ: ปัจจุบันตัวชีวัดทางชีวภาพจากการตรวจซีรั่มครีเอทินีน และอัลบูมินในปัสสาวะมีความไวต่ำในการประเมินความเสี่ยงต่อโรคไต การใช้ตัวบ่งชี้ทางชีวภาพในปัสสาวะกลุ่มใหม่จากการที่ท่อไตถูกทำลาย (urine biomarker of tubular injury) อาจสามารถช่วยทำนายในผู้ป่วยโรคเบาหวานชนิดที่ 2 ที่มีความเสี่ยงสูงต่อโรคไต การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาความสามารถของตัวบ่งชี้ทางชีวภาพในปัสสาวะในการพยากรณ์การเสื่อมของไตและผลลัพธ์ร่วมของการเสื่อมของไตและการเสียชีวิตในผู้ป่วยโรคเบาหวานชนิดที่ 2

ระเบียบวิธีวิจัย: เป็นการศึกษาไปข้างหน้าในผู้ป่วยโรคเบาหวานชนิดที่ 2 จำนวน 257 ราย มีการเก็บตัวอย่างตรวจตัวบ่งชี้ทางชีวภาพในปัสสาวะจากการบาดเจ็บของท่อไตเมื่อเริ่มต้นการศึกษา โดยมีผลลัพธ์ของการศึกษาคือ ผลลัพธ์ร่วมของการเกิดโรคไตคือ โรคไตเรื้อรังระยะสุดท้าย การลดลงของอัตราการกรองของไตมากกว่าเท่ากับร้อยละ 40 และการเสียชีวิต

ผลการศึกษา: ผู้ป่วยส่วนใหญ่มีโรคไตเรื้อรังระยะที่ 3-4 และมีค่ามัธยฐานของระดับอัลบูมิน/คริเอทินีนในปัสสาวะ 60.8 มก./กรัม ค่ามกลางของระยะเวลาการติดตามผู้ป่วยประมาณ 7 ปี ระดับของ cystatin-C, angiotensinogen, kidney injury molecule-1 (KIM-1) และ neutrophil-gelatinase associated lipocalin (NGAL) ในปัสสาวะเพิ่มขึ้นในผู้ป่วยที่เกิดผลลัพธ์ร่วมของการเกิดโรคไต ระดับของตัวบ่งชี้ทางชีวภาพในปัสสาวะทุกตัวมีความสามารถปานกลางในการพยากรณ์ผลลัพธ์ทางไตโดยมี area under the curve (AUC) อยู่ที่ 0.65-.072 ซึ่งใกล้เคียงกับระดับอัลบูมิน/คริเอทินีนในปัสสาวะ เมื่อใช้เกณฑ์ตัดที่เหมาะสมที่สุด (optimal cut-off values) สำหรับตัวบ่งชี้ทางชีวภาพในปัสสาวะแต่ละชนิดพบว่า ระดับที่สูงมีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับการเกิดผลลัพธ์ร่วมของการเกิดโรคไต อย่างไรก็ตามหลังการปรับปัจจัยอื่นที่มีผลต่อไต แล้วใช้ Cox proportional hazards model พบว่า ระดับตัวบ่งชี้ทางชีวภาพในปัสสาวะเพียงของ cystatin-C และ KIM-1 สามารถทำนายการเกิดผลลัพธ์ร่วมของการเกิดโรคไตได้อย่างมีนัยสำคัญทางสถิติ

สรุป: ระดับของตัวบ่งชี้ทางชีวภาพในปัสสาวะสามารถพยากรณ์ผลลัพธ์ร่วมของการเกิดโรคไตและการเสียชีวิตในผู้ป่วยเบาหวานชนิดที่ 2 ได้

คำสำคัญ: ตัวบ่งชี้การเสื่อมของไต; ไตวาย; โรคไตเรื้อรัง; ไตเสื่อม; อัลบูมินในปัสสาวะ; โปรตีนรั่ว

Introduction

The global prevalence of type 2 diabetes mellitus (T2DM) in 2017 was 451 million cases, and it is estimated to increase to 693 million cases by 2045.[1] According to the 2020 Thailand Renal Replacement Therapy Registry Report, the primary etiology of end-stage kidney disease was diabetic nephropathy. Albuminuria is generally considered an early and non-invasive biomarker for the development of nephropathy.[2] However, urine albuminuria is not always indicative of kidney injury, and a significant proportion of renal impairment occurs before the onset of albuminuria. Additionally, microalbuminuria can revert back to normoalbuminuria in diabetic nephropathy.[3-5] Therefore, identifying more sensitive and specific biomarkers that reflect kidney injury would be beneficial for identifying diabetic patients at risk of renal progression.

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Diabetic nephropathy affects the glomerular endothelium, mesangial cells, podocytes, and tubular epithelium.[6] Changes in renal pathology are observed before the onset of albuminuria.[7] Recently, alterations in the tubulointerstitium have been increasingly recognized in the pathogenesis of diabetic nephropathy. Tubulointerstitial damage has been demonstrated to predict renal survival in both diabetic and nondiabetic glomerular diseases.[8-9] As diabetic nephropathy progresses, tubulointerstitial damage intensifies, and the ability to reabsorb low molecular weight proteins further diminishes. Urine markers such as cystatin-C, angiotensinogen, kidney injury molecule-1 (KIM-1), and neutrophil-gelatinase associated lipocalin (NGAL) are almost completely filtered by the glomeruli and predominantly reabsorbed in the renal tubules, akin to other low molecular weight proteins.[10-17] Due to these observations, urine biomarkers indicative of tubular injury may offer improved sensitivity in predicting renal progression in diabetic nephropathy.[18]

Methods

Study population

Patients with T2DM from the outpatient clinic of Phramongkutklao Hospital between February 2014 and February 2015 were recruited. The inclusion criteria comprised age ≥18 years and an estimated glomerular filtration rate (eGFR) of ≥15 ml/min/1.73 m2. Exclusion criteria encompassed acute kidney injury, pregnancy, and a life expectancy of less than 1 year. These patients were followed prospectively for a minimum of 60 months. The study received approval from the Ethics Committee of the Institute Review Board of the Royal Thai Army Medical Department and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Laboratory Data and Urine Biomarkers

Laboratory data were collected both at baseline and at the study's conclusion. Fasting blood samples were drawn in the morning. The estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.19 Urine biomarkers were assessed at baseline. Thirty milliliters of fresh urine were centrifuged at 4,000 rpm for 10 minutes to remove particulate impurities. Subsequently, the supernatant was stored frozen at -80°C until assayed. All renal tubular biomarkers were determined using a commercially available quantitative sandwich ELISA technique, following the manufacturer's instructions (angiotensinogen and cystatin C by R&D Systems China Co., Ltd., and NGAL and KIM-1 by R&D Systems Inc., USA & Canada).

Outcome

The outcomes comprised the composite outcome of end-stage kidney disease (ESKD), a 40% decline in eGFR, and death.

Statistical analyses

Descriptive statistics were presented as means ± standard deviation, median (interquartile range), or frequency (percentage). The Student's t-test was employed to compare continuous variables, while the chi-square test was utilized for categorical variables. Sensitivity, specificity, and the area under the curve (AUC) were computed as indicators of diagnostic accuracy. Receiver operating characteristic (ROC) curves were employed for AUC calculations. A perfect test exhibits an area under the ROC curve of 1.0, whereas a flawed test would have a value of ≤0.5. Kaplan-Meier curves were constructed to evaluate outcomes using the ROC-derived cut-off level for each tubular biomarker. Multivariate Cox proportional hazard regression analysis was conducted to determine factors associated with the outcomes. A p-value <0.05 was deemed statistically significant. The data were analyzed using the SPSS 16.0 statistical software program (SPSS, Chicago, IL, USA).

Results

Two hundred fifty-seven patients were included in the study and followed prospectively for a minimum of 84 months. Outcome data were available for 78.5% of these patients. The cumulative incidence of the composite outcome (ESKD, a 40% decline in eGFR, and death) occurred in 88 patients (34.2%). Specifically, 40 patients (15.6%) developed ESKD, while 21 (8.2%) patients passed away. The baseline characteristics of patients, categorized by the occurrence of the composite renal

outcome, are presented in Table 1. The majority of patients exhibited baseline albuminuria levels exceeding 30 mg/day, with 34.6% having microalbuminuria and 28.8% having macroalbuminuria. Furthermore, 56.8% of patients had an eGFR below 60 ml/min/1.73 m2. Patients who met the composite renal outcome criteria exhibited a significantly higher prevalence of cardiovascular disease, advanced CKD, and anemia. Additionally, elevated baseline systolic blood pressure, intact parathyroid hormone levels, serum phosphate levels, urine albumin, and all urine tubular biomarkers, coupled with a reduced baseline GFR, were significantly observed in patients with positive composite renal outcomes.

Table 1. Baseline characteristics of all patients and according to the composite renal outcome

Parameters	All patients (n = 257)	Positive composite renal outcome (n = 88)	Negative composite renal outcome (n =169)	P
Age (year)	66.22 ± 10.63	67.08 ± 9.47	65.78 ± 11.18	0.351
Male (n,%)	139 (54.1%)	49 (55.7%)	90 (53.3%)	0.711
Duration of DM (years)	10 (5, 20)	10 (8, 20)	10 (5, 20)	0.156
Duration of FU (years)	7 (5.28, 7)	3.32 (1.67, 5.62)	7 (7, 7)	<0.001
Stages of CKD				<0.01
CKD I	39 (15.2%)	4 (4.5%)	35 (20.7%)	
CKD II	72 (28%)	16 (18.2%)	56 (33.1%)	
CKD III	100 (38.9%)	39 (44.3%)	61 (36.1%)	
CKD IV	46 (17.9%)	29 (33%)	17 (10.1%)	
Hypertension (n,%)	244 (94.9%)	84 (95.5%)	160 (94.7%)	0.787
Dyslipidemia (n,%)	238 (92.6%)	79 (89.8%)	159 (94.1%)	0.210
CVD (n,%)	10 (3.9%)	7 (8%)	3 (1.8%)	0.015
Anemia (%)	127 (49.4%)	59 (67%)	68 (40.2%)	<0.001
Medications				
ACEI or ARB (n/%)	200 (77.8%)	71 (80.7%)	129 (76.3%)	0.420
Insulin (n/%)	61 (23.7%)	25 (28.4%)	36 (21.3%)	0.204
Aspirin (n/%)	158 (61.5%)	58 (65.9%)	100 (59.2%)	0.290
Systolic BP (mmHg)	139.4 ± 18.6	144.1 ± 20.41	136.9 ± 17.14	0.003
Diastolic BP (mmHg)	76.69 ± 11.58	76.03 ± 11.35	77.03 ± 11.71	0.514
BMI (kg/m^2)	27.11 ± 4.83	27.01 ± 5.29	27.17 ± 4.59	0.807
Labs				
eGFR (mL/min/1.73m^2)	57.15 ± 26.31	43.43 ± 21.86	64.29 ± 25.64	<0.001
Urine alb/Cr (mg/g)	60.8 (10.7, 325.4)	375.75 (62.7, 1540.7)	37.1 (7.8, 138.4)	<0.001
FPG (mg/dL)	142.54 ± 58.65	146.9 ± 66.78	140.25 ± 53.98	0.393
HbA1c (%)	7.29 ± 1.49	7.47 ± 1.85	7.2 ± 1.28	0.245
Hemoglobin (g/dL)	12.21 ± 1.62	11.71 ± 1.72	12.58 ± 1.44	0.001

Parameters	All patients (n = 257)	Positive composite renal outcome (n = 88)	Negative composite renal outcome (n =169)	P
Phosphate (mg/dL)	3.42 ± 0.63	3.57 ± 0.66	3.32 ± 0.58	0.020
Intact-PTH (pg/mL)	71.96 (54.31, 171.2)	120.5 (59.46, 224.3)	57.65 (41.34, 69.47)	0.007
Urine biomarkers				
UCCR (mcg/gm)	2.95 (0.99, 6)	4.99 (2, 9.35)	2.46 (0.6, 4.08)	<0.001
UANG (mcg/gm)	2.21 (0.28, 9.65)	5.15 (0.95, 18.11)	1.51 (0.22, 4.69)	<0.001
UKIM-1 (ng/gm)	66.21 (30.58, 124.43)	107.3 (57.81, 191.57)	52.39 (20.61, 94.18)	<0.001
UNGAL (ng/gm)	681.4 (406.7, 1216)	895 (548.6, 1546)	601 (360.4, 1014)	<0.001

DM, diabetes mellitus; FU follow-up; CKD, chronic kidney disease; CVD, cardiovascular disease; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, hemoglobin A1C; PTH, parathyroid hormone; urine alb/cr, urine albumin/creatinine; UCCR, urine cystatin C; UANG, urine angiotensinogen; UKIM-1, urine KIM-1; UNGAL, urine NGAL

The levels of urine biomarkers corresponding to the composite renal endpoint are depicted in **Figure 1**. All tubular biomarker levels were higher in the group of patients who reached the composite renal endpoint compared to those who did not. The median values of urine biomarkers, adjusted for 1 gram of creatinine, for the group with the composite renal endpoint versus the group without were as follows: cystatin C 4.99 (2–9.35) vs. 2.46 (0.6-4.08) mcg/gCr, P=0.001; angiotensinogen 5.15 (0.95–18.11) vs. 1.51 (0.22-4.69) mcg/gCr, P=0.001; KIM-1 107.34 (57.81–191.57) vs. 52.39 (20.61-94.18) ng/gCr, P=0.001; NGAL 895.01 (548.6–1,546.21) vs. 601.02 (360.36-1,014.07) ng/gCr, P=0.001.

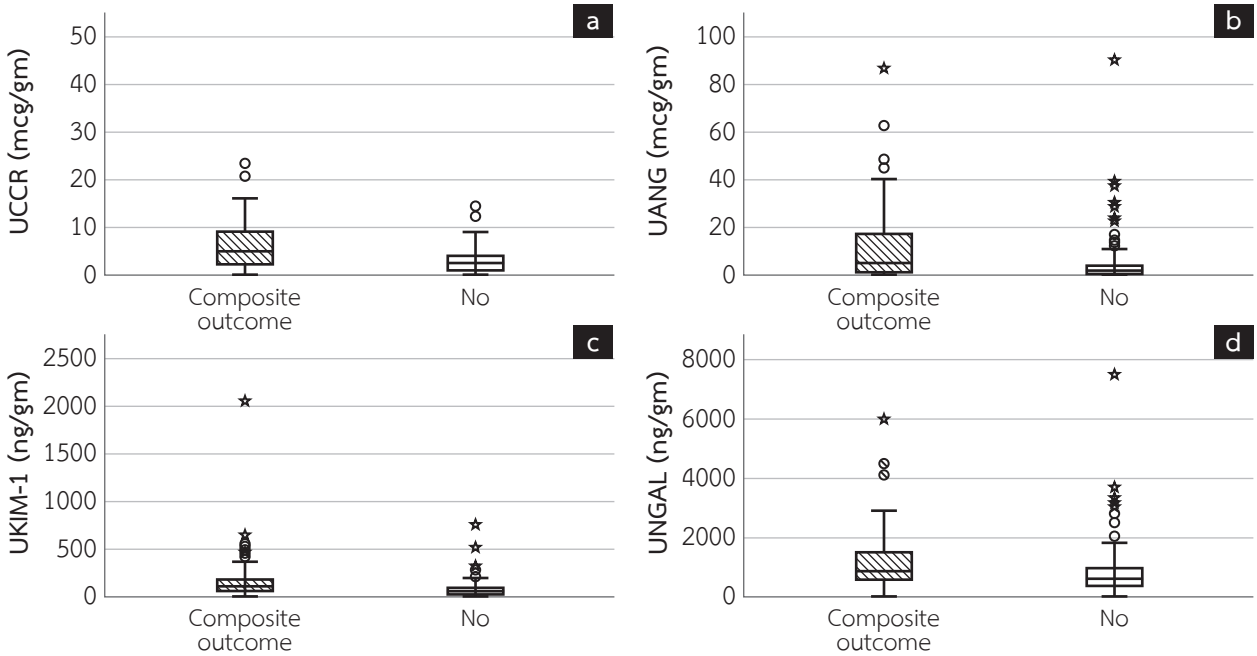

Figure 1. The levels of urine biomarkers corresponding to the composite renal endpoint are:
a) Cystatin-C; b) Angiotensinogen; c) KIM-1; and d) NGAL, adjusted for 1 gram of urine creatinine.

ROC curve analyses, showing the AUC for each urine biomarker concerning the composite renal endpoint, are presented in **Table 2** and **Figure 2**. The optimal cut-off values for urine biomarkers were as follows: cystatin-C at 3.35 mcg/g (sensitivity 67.0%, specificity 70.2%), angiotensinogen at 3.5 mcg/g (sensitivity 64.8%, specificity 70.8%), KIM-1 at 71 ng/g (sensitivity 70.5%, specificity 64.3%), and NGAL at 473 ng/g (sensitivity 85.2%, specificity 39.3%). All tubular biomarkers exhibited intermediate performance in predicting the composite renal endpoint and were comparable to urine albumin/creatinine. Combining urine biomarkers of tubular injury with urine albumin/creatinine slightly enhanced the performance, as shown in **Table 2** and **Figure 3**.

Table 2 AUC of urine biomarkers in predicting the renal endpoint

Urine biomarkers	AUC	95% confidence interval		P-value
		Lower	Upper	
UCCR (mcg/gm)	0.704	0.636	0.773	<0.001
UANG (mcg/gm)	0.698	0.628	0.767	<0.001
UKIM-1 (ng/gm)	0.721	0.657	0.785	<0.001
UNGAL (ng/gm)	0.646	0.578	0.715	<0.001
UACR	0.667	0.587	0.747	<0.001
UACR+UCCR	0.732	0.665	0.8	<0.001
UACR+UANG	0.719	0.649	0.788	<0.001
UACR+UKIM-1	0.747	0.682	0.812	<0.001
UACR+UNGAL	0.667	0.587	0.747	<0.001

UACR, urine albumin/creatinine; UCCR, urine cystatin-C; UANG, urine angiotensinogen; UKIM-1, urine KIM-1; UNGAL, urine NGAL

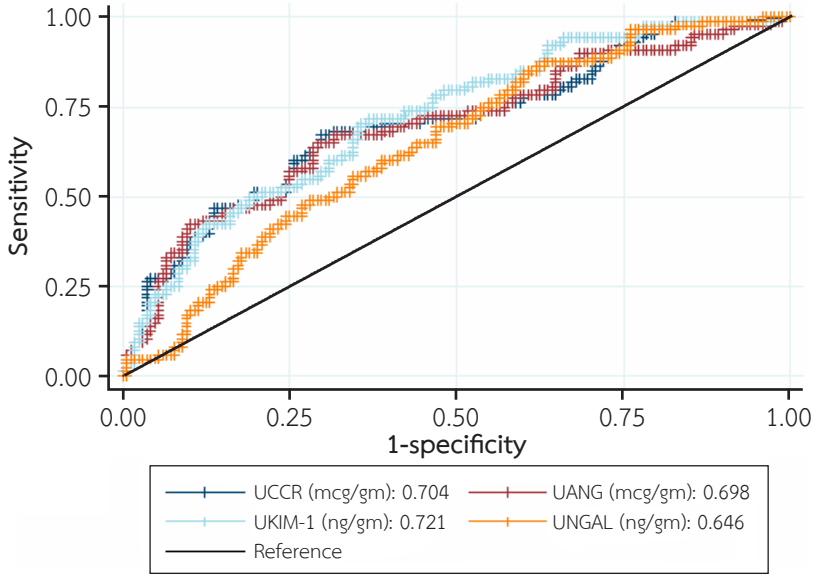

Figure 2. ROC curve showing the AUC for each urine biomarker in predicting the composite renal endpoint. *UCCR, urine cystatin-C; UANG, urine angiotensinogen; UKIM-1, urine KIM-1; UNGAL, urine NGAL*

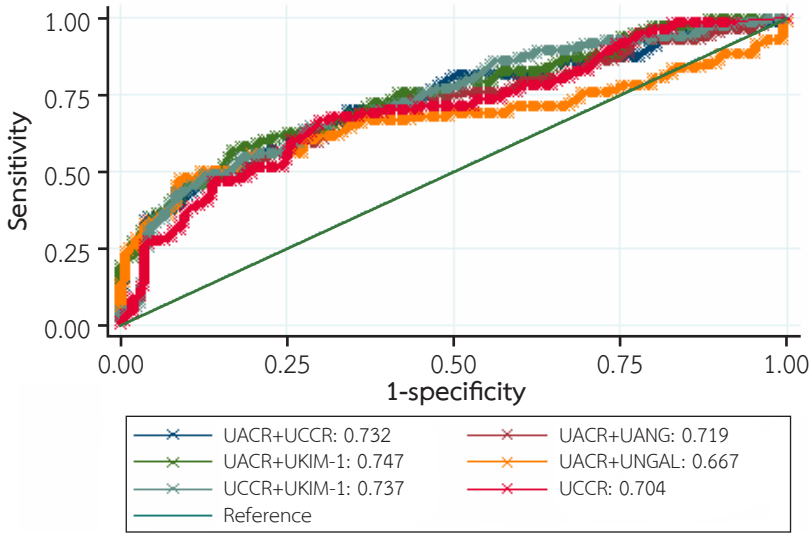

Figure 3 AUC of the combined urine biomarkers of tubular injury and urine albumin/creatinine for predicting the composite renal endpoint

UACR, urine albumin/creatinine; UCCR, urine cystatin-C; UANG, urine angiotensinogen; UKIM-1, urine KIM-1; UNGAL,

Kaplan-Meier survival curves for the composite renal outcome, encompassing a 40% decline in eGFR, ESKD, and death using the optimal cut-off values of urine biomarkers, are depicted in **Figure 4**. For the composite renal endpoint, the optimal cut-off values for urine biomarkers were as follows: cystatin-C at 3.35 ng/gCr, angiotensinogen at 3.5 mcg/gCr, KIM-1 at 71 ng/gCr, and NGAL at 473 ng/gCr. Patients with all urine tubular biomarker levels exceeding the optimal cut-off values were found to develop the composite renal endpoint (P<0.001).

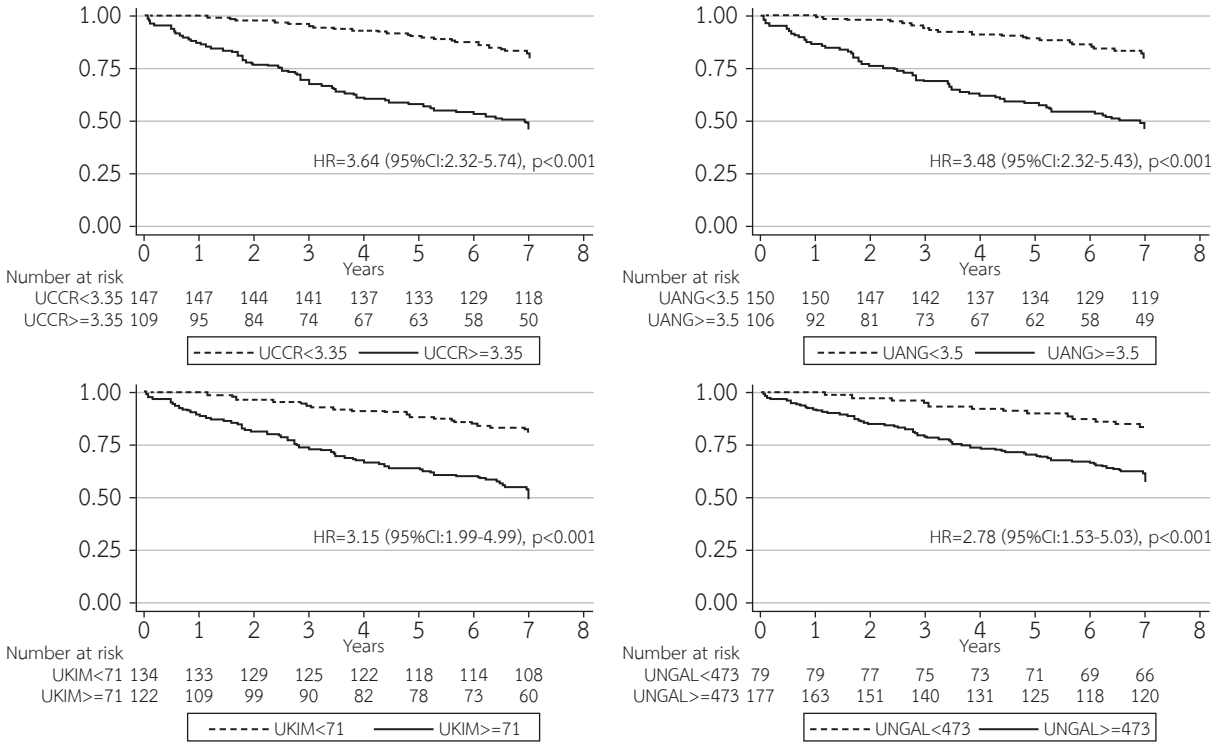

Figure 4. Kaplan-Meier curves for the composite renal outcome using the optimal cut-off value for each urine biomarker

UCCR, urine cystatin-C; UANG, urine angiotensinogen; UKIM-1, urine KIM-1; UNGAL, urine NGAL

The Cox proportional hazards model for the composite renal endpoint among the quartiles of urine tubular biomarker levels is presented in **Table 3**. After adjusting for relevant risk factors for renal progression, only the upper quartiles of urine cystatin-C (adjusted HR 2.38; 95% CI 1.13 to 5.33) and KIM-1 (adjusted HR 3.12; 95% CI 1.62 to 6.03) significantly predicted the composite renal endpoint.

Table 3. Cox proportional hazards model for the composite renal endpoint

Urine Biomarkers	UCCR HR [95%CI]	P	UANG HR [95%CI]	P	UKIM-1 HR [95%CI]	P	UNGAL HR [95%CI]	P
Unadjusted								
1st quartile	Reference		Reference		Reference		Reference	
2nd quartile	1.01 (0.43, 2.39)	0.98	0.47 (0.2, 1.13)	0.09	1.34 (0.6, 3)	0.47	1.06 (0.45, 2.49)	0.9
3rd quartile	2.61 (1.19, 5.71)	0.02	2.05 (0.99, 4.22)	0.05	1.23 (0.54, 2.79)	0.62	2.67 (1.25, 5.68)	0.01
4th quartile	3.01 (1.38, 6.53)	<0.01	1.39 (0.66, 2.92)	0.38	3.69 (1.68, 8.06)	<0.01	1.76 (0.79, 3.94)	0.17
Adjusted*								
1st quartile	Reference		Reference		Reference		Reference	
2nd quartile	1 (0.51, 1.99)	0.99	0.95 (0.49, 1.84)	0.88	1.04 (0.53, 2.02)	0.91	0.84 (0.42, 1.67)	0.62
3rd quartile	3.15 (1.61, 6.16)	<0.01	4.67 (2.39, 9.16)	<0.01	1.58 (0.83, 3.01)	0.17	2.59 (1.4, 4.77)	<0.01
4th quartile	2.38 (1.13, 5.03)	0.02	1.24 (0.55, 2.82)	0.61	3.12 (1.62, 6.03)	<0.01	1.03 (0.47, 2.24)	0.94

**Adjusted for baseline factors including age, sex, body mass index, systolic blood pressure, hypertension, dyslipidemia, cerebrovascular accident, use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers, estimated glomerular filtration rate and urine albumin/creatinine*

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

Discussion

The main findings of the present study indicate that urine biomarkers of tubular injury, including cystatin-C, angiotensinogen, KIM-1, and NGAL, were significantly associated with the composite outcome of ESKD, a 40% decline in eGFR, and death in T2DM patients with varying degrees of renal impairment. The higher quartiles of urine cystatin C and KIM-1 were significantly associated with the renal outcome. Using the optimal cut-off value for each urine biomarker derived from ROC analyses, the performance of urine biomarkers was comparable to that of urine albumin/creatinine in predicting the renal outcome. Combining urine biomarkers with urine albumin/creatinine performed slightly better than using urine biomarkers alone.

Research on more sensitive biomarkers for predicting

renal progression in diabetic nephropathy is ongoing. The pathogenesis of diabetic nephropathy involves genetic, environmental, inflammatory, and biological factors. Novel urine biomarkers are either directly or indirectly related to these mechanisms.[17] Several studies have confirmed the significant role of tubular injury and tubulointerstitial fibrosis in the progression of kidney disease, irrespective of its etiology.[20] These novel biomarkers of tubular injury may prove useful in predicting renal progression and long-term prognosis. Urine biomarkers such as cystatin-C, angiotensinogen, KIM-1, and NGAL indicate tubular injury and are beneficial for the early detection of acute kidney injury before glomerular damage or albuminuria develops.[21-24] They can also reflect the decline in GFR and the extent of tubulointerstitial fibrosis.[3] The present study suggested that higher levels of urine biomarkers of tubular damage at baseline were associated with worsened renal outcomes, although other studies have reported conflicting results.[24,25]

Cystatin-C is easily filtered by the glomeruli and subsequently reabsorbed and catabolized by the proximal tubules. Elevated urine cystatin-C levels have been recognized as markers of renal tubular dysfunction.[26] Previous prospective observational studies have shown that, along with albuminuria, urine cystatin-C is significantly associated with the annual decline in eGFR in patients with T2DM.[18,27] Our results similarly indicated that urine cystatin-C serves as an independent predictor of CKD progression.

NGAL is expressed in the renal tubular epithelium, and its urinary concentration increases following acute renal injury.[16] Overexpression of NGAL has been detected in distal tubular cells in CKD, and the level of urinary NGAL correlates with the severity of nephropathy.[28,29] This could be attributed to the release of NGAL from renal tubules in response to metabolic and hemodynamic stress. A previous observational study indicated that urine NGAL could predict albuminuria and the progression of diabetic nephropathy.[30] Other studies have reported consistent findings.[18,23] Similarly, our study found that urine NGAL predicted a worsening CKD outcome; however, after adjusting for relevant CKD risk factors, the upper quartiles of urine NGAL did not significantly predict the composite renal endpoint.

The expression of KIM-1 increases on the membrane of proximal tubule cells in response to inflammation and fibrosis.[31] Other studies have shown that urine KIM-1 levels were significantly associated with the decline in eGFR among patients with T1DM, vasculitis, and glomerulonephritis.[32,33] However, urine KIM-1 was not associated with the decline in renal function in other types of CKD.[34] The present study underscores the capability of urine KIM-1 in predicting CKD progression in T2DM. The variability in findings across studies could stem from differences in the etiology of renal disease and underlying pathology.

An increase in urine angiotensinogen has been observed in patients with elevated renal angiotensin II levels. Treatment with an angiotensin receptor blocker has been demonstrated to reduce urine angiotensinogen levels.[35] The utility of urine angiotensinogen in assessing intrinsic renal angiotensin II activity correlates with the extent of tubulointerstitial damage in diabetic nephropathy.[24] The results from our study on urine angiotensinogen align with those of other studies.[18,36]

The present study found that the ability of novel markers of tubular injury to predict CKD progression in patients with T2DM was comparable to that of conventional urine albumin/creatinine. This might be attributed to the fact that most patients in this study had significant albuminuria and impaired renal function at baseline (albuminuria >30 mg/day and eGFR <60 ml/min/1.73 m^2). Therefore, the potential utility of urine biomarkers in patients without albuminuria should not be disregarded, and further studies are warranted.

There are several limitations to this study. The population was derived from a single center, potentially limiting the generalizability of the results. Additionally, most patients were in the later stages of CKD with significant albuminuria, which might mask the ability of urine biomarkers to predict renal outcomes in earlier-stage patients. Furthermore, the concentrations of urine biomarkers might reflect damage to the

filtration barrier rather than tubular damage due to biomarker accumulation in the circulation in advanced CKD.[37] Plasma levels of these biomarkers were also not assessed in this study. Nonetheless, previous research has affirmed the utility of these urine biomarkers in early prediction of renal injury before any discernible changes in GFR occur. Given that these biomarkers are freely filtered by the glomerulus and reabsorbed in the proximal tubules, their appearance in urine suggests direct renal injury. Experimental evidence indicates that elevated levels of NGAL and KIM-1 may stem from impaired reabsorption in the proximal tubules.[38,39] The results of this study may also imply that urine biomarker levels reflect damage to both glomeruli and tubules.

Conclusion

The urine biomarkers of tubular damage were significantly associated with the composite outcome of ESKD, a 40% decline in eGFR, and death in T2DM patients with varying degrees of renal impairment and albuminuria. However, the performance of these urine biomarkers in predicting renal outcomes was no better than that of urine albumin/creatinine. Further studies are necessary to evaluate the predictive ability of these urine biomarkers for renal progression in type 2 diabetic patients at earlier stages of CKD and without significant albuminuria.

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