

Original Article

Low plasma uromodulin is a predictor of early stage chronic kidney disease progression

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Abstract: Uromodulin might play an important role in maintaining normal kidney function. However, the relationship between uromodulin concentration and the clinical outcomes of patients with chronic kidney disease (CKD) is poorly known. The aim of the present study was to clarify whether plasma uromodulin concentration is associated with renal outcomes in CKD patients. This prospective observational study included 116 patients with CKD (non-end-stage renal disease) and 40 healthy controls. ELISA was performed to measure the concentration of plasma uromodulin in all the participants. The association between plasma uromodulin and estimated GFR (eGFR) was then evaluated. Uromodulin concentrations of CKD patients were divided into quartiles. Survival and Cox analysis were utilized to determine the risk of reduced plasma uromodulin for predicting the poor renal outcomes. The expression level of plasma uromodulin was significantly reduced in patients with CKD compared with the healthy volunteers ($P < 0.01$). A positive correlation was found between plasma uromodulin and eGFR. In addition, the CKD patients with lower plasma uromodulin have a higher chance to develop into end-stage renal disease (ESRD) ($P < 0.01$). Moreover, the CKD patients in the 3rd and 4th plasma uromodulin quartiles (higher plasma uromodulin concentrations) had significantly better renal outcomes than those in the 1st quartile (lower plasma uromodulin concentrations). Taken together, lower plasma uromodulin levels were associated with poorer renal outcome, indicating that uromodulin may be a useful biomarker for predicting the progression of CKD.

Keywords: Uromodulin, chronic kidney disease, renal outcome, end-stage renal disease

Introduction

Chronic kidney disease (CKD) is defined as sustained kidney damage indicated by the presence of structure and/or function abnormalities (microalbuminuria/proteinuria, hematuria, histologic or imaging abnormalities etc), or as an unexplained reduction in glomerular filtration rate (GFR) to less than 60 mL/1.73 m² over 3 months [1]. Based on estimated GFR (eGFR), CKD has been categorized into five stages (CKD 1-5). The major outcomes of CKD include end-stage renal disease (ESRD) and the development and progression of cardiovascular disease (CVD) [2-4]. Identifying the circulating biomarkers that are closely associated with kidney function and the progression of CKD can not only help monitor therapeutic effects in real-time, but also contribute to early detection and diagnosis of CKD.

Uromodulin, also known as TammHorsfall protein, is a 95 kDa protein encoded by the UMOD gene located on chromosome 16p12.3.1 [5]. Under normal physiological situation, it has been demonstrated to play important role in protecting against bacterial urinary tract infection and preventing kidney stone formation. In addition, uromodulin might also involve in regulating tubular water impermeability, salt support and inflammation [6, 7]. Urinary excretion of uromodulin is usually decreased in parallel with the GFR. In addition, negative association was found between uromodulin and tubular atrophy, indicating that uromodulin might be an active player in CKD progression. However, different results were reported regarding the relationship between serum uromodulin concentrations and GFR. Prajczek et al showed that eGFR correlated negatively with serum uromodulin [8]. Risch et al revealed that serum uromodulin had inverse relationships with creatinine, cys-

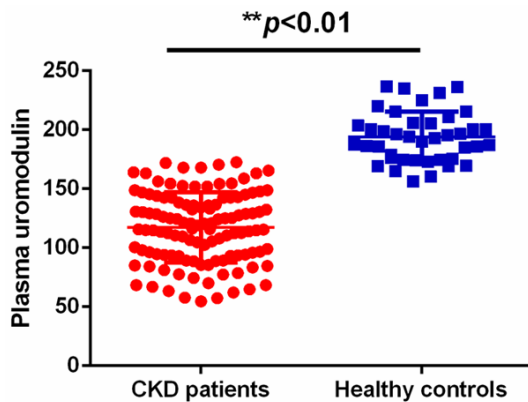


Figure 1. The concentrations of plasma uromodulin in patients with CKD and healthy volunteers.

tatin C, and urea as well as a positive relationship with eGFR [9].

Although uromodulin might play an important role in regulating normal kidney function, little information is known regarding whether plasma uromodulin expression level can be used to predict the prognosis and progression of CKD that diagnosed at the early stage. Therefore, the purpose of this study was to elucidate the potential clinical significance of plasma uromodulin in CKD.

Materials and methods

Study population

One hundred and sixteen patients and 40 healthy volunteers were recruited in the study. The study was approved by the Ethics committee of the First Affiliated Hospital of Shantou University Medical College, and written informed consent was obtained from all participants. All enrolled patients were with stage 1-2 CKD and they had relatively conserved renal function ($\text{eGFR} \geq 60 \text{ mL/min/1.72 m}^2$). The patients with eGFR less than 60 were excluded from the study. The Modification of Diet in Renal Disease Study (MDRD) equation was used to estimate GFR. In addition, serum creatinine $< 1.2 \text{ mg/dL}$ and no history of administration of renin-angiotensin system (RAS) were required. The exclusion criterion were as following: active urinary tract infection; renal disease other than diabetic nephropathy; neoplastic disorders; severe liver dysfunction; active or chronic infection or inflammatory disorders; pregnancy; or a recent [within 6 months] history of acute myocardial infarction, stroke, or occlusive peripheral vascular disease).

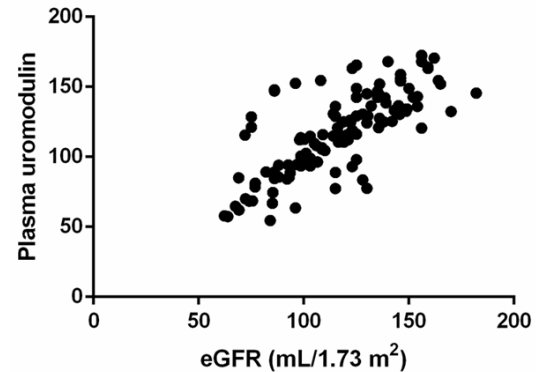


Figure 2. The association between plasma uromodulin and eGFR.

Uromodulin immunoassay

Up to 5 mL of venous blood was collected from each participant. Then the plasma was prepared by centrifugation at $2000 \times g$ for 30 min at 4°C . Then samples were divided into aliquots in cryovials and stored at -80°C until analysis. The plasma concentrations of uromodulin were analyzed by rabbit anti-human uromodulin antibody (3 g/mL; Biotrend, Cologne, Germany) as previously described [10].

Statistically analysis

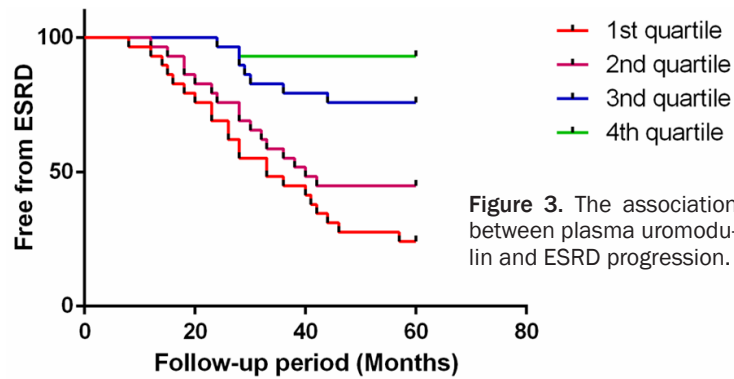
Statistical differences in plasma uromodulin in CKD versus control patients were tested using the non-parametric Mann-Whitney test. Correlations were tested using the Spearman's tests. Survival curves were estimated by the Kaplan-Meier method and evaluated by the log-rank test. Cox analysis was performed to evaluate the association of lower quartiles of uromodulin with renal outcomes. The highest quartile of plasma uromodulin was used as the reference category. The interaction effects of uromodulin and other variables on outcomes were estimated by adding interaction terms between the status of uromodulin and that of other variables to the relevant model. All statistical analyses were performed using SPSS 21.0 software (SPSS, Chicago, IL.), with a P value less than 0.05 indicating statistical significance.

Results

The concentrations of plasma uromodulin in patients with CKD and healthy volunteers

The ELISA results showed the concentrations of plasma uromodulin was significantly reduced

The clinical value of plasma uromodulin for CKD



lower plasma uromodulin have a higher chance to develop into ESRD, suggesting that plasma uromodulin was a promising biomarker for predicting the prognosis of early stage CKD ($P < 0.01$) (Figure 3).

Hazard ratios for renal outcomes according to quartiles of plasma uromodulin

Univariate analysis was performed to evaluate the risk factors for patients with CKD and the results were summarized in Table 1. Table 2 showed the hazard ratios for poor renal outcomes according to quartiles of plasma uromodulin. After adjustments for demographic, traditional and nontraditional cardiovascular risk factors, use of immunosuppressants and renin-angiotensin-aldosterone system inhibitors, ischemic heart disease, the CKD patients in the 3rd and 4th plasma uromodulin quartiles had significantly better renal outcomes than those in the 1st quartile.

Discussion

CKD has become a significant public health problem and the increasing number of CKD in recent years is due to the higher incidence of non-communicable diseases, especially diabetes and hypertension [11-13]. It remains asymptomatic till late stage when intervention become ineffective to deter the progression of the disease [14]. Therefore, it is urgent and important to explore biomarkers that can early diagnosis as well as predict the prognosis of this deadly disease. Biopsy is considered the gold standard for definitive diagnosis. However, it is impossible to use tissue biopsy repetitively to monitor the disease stages, therapeutic responses and prognosis in a real time setting. Body fluids such as saliva, serum, plasma, and urine have become valuable tools for detecting innovative biomarkers.

Our results showed that plasma uromodulin expression was significantly decreased in CKD patients in comparison with the controls. In addition, it was positively correlated with eGFR. Moreover, the CKD patients with lower plasma uromodulin have a higher chance to progress into ESRD as well as lead to poorer clinical out-

Table 1. Univariate analysis of risk factors for renal outcomes in patients with CKD

Variables	HR	95% (CI)	P
Age (per 10 years increase)	1.29	1.04-1.56	<0.01
Male	1.16	0.95-1.32	0.12
Smoking	1.76	1.35-2.38	<0.01
Diabetes mellitus	3.28	2.36-4.19	<0.01
Systolic blood pressure	1.45	1.13-1.74	0.01
Dyslipidemia	1.92	1.44-2.53	<0.01
Immunosuppressants	0.36	0.15-0.58	<0.01
RAAS inhibitors	2.41	1.95-2.81	<0.01
Daily proteinuria	1.12	0.96-1.48	0.08
Hemoglobin	0.74	0.54-0.91	<0.01
Serum albumin	0.29	0.18-0.45	<0.01
Serum phosphorus	2.93	2.01-3.84	<0.01
Ischemic heart disease	2.12	1.72-2.64	<0.01

in patients with CKD in comparison with the healthy controls ($P < 0.01$) (Figure 1, Supplementary Table).

The association between plasma uromodulin and eGFR

The correlation analysis showed that there was a positive association between plasma uromodulin and eGFR ($r = 0.7531$, $P < 0.01$) (Figure 2), indicating that the concentrations of plasma uromodulin might be closely related with eGFR.

The association between plasma uromodulin and ESRD development

All 116 patients were divided into 4 quartiles based on the concentrations of plasma uromodulin. The CKD patients in 1st quartile had lowest concentrations of plasma uromodulin while those in the 4th quartile had highest concentrations of plasma uromodulin. The survival analysis revealed that the CKD patients with

Table 2. Hazard ratios (HRs) for renal outcomes according to quartiles of plasma uromodulin

	Model 1 (Beta = -0.039)			Model 2 (Beta = -0.072)			Model 3 (Beta = -0.006)			Model 4 (Beta = -0.023)		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
1st quartile	reference			reference			reference			reference		
2nd quartile	0.84	0.65-1.13	0.08	0.88	0.67-1.23	0.20	0.94	0.72-1.46	0.31	0.91	0.74-1.38	0.26
3rd quartile	0.52	0.32-0.81	<0.01	0.61	0.45-0.89	<0.01	0.84	0.63-1.08	0.12	0.82	0.61-1.03	0.07
4th quartile	0.28	0.14-0.63	<0.01	0.33	0.19-0.70	<0.01	0.43	0.32-0.76	<0.01	0.45	0.33-0.71	<0.01

Model 1: adjustments for age and sex. Model 2: model 1 plus adjustments for smoking, diabetes mellitus, systolic blood pressure, and dyslipidemia. Model 3: model 2 plus adjustments for use of immunosuppressants, use of RAAS inhibitors, daily proteinuria, hemoglobin, eGFR, serum albumin, and serum phosphorus. Model 4: model 3 plus adjustments for ischemic heart disease.

come. To the best of our knowledge, this was the first time to demonstrate that early stage CKD patients with lower plasma uromodulin have adverse clinical outcome for developing into ESDR. Taken together, our data indicated that plasma uromodulin may be a useful biomarker for predicting the CKD development. Consistent with our results, Steubl et al showed that plasma uromodulin was closely associated with had better performance for differentiation of subjects with CKD stage 0 and CKD stage I compared with creatinine, cystatin C, BUN, and eGFR, indicating that plasma uromodulin can help identify CKD in an early clinical stage [15]. Risch et al reported that serum uromodulin displayed lower concentrations with decreasing kidney function, as it could reflect a reduction in number or function of these cells during the progression of CKD [9]. Prajczet et al showed that a positive relation was found between serum uromodulin tumors necrosis factor- α , interleukin-6 (IL-6), IL-8 and IL-1. Persistent chronic thick ascending limb (TAL) damage led to a reduction in the number of TAL cells and an attenuation of urinary and serum uromodulin concentrations, indicating uromodulin played an active role in the development of CKD [8]. Zhou et al demonstrated that urinary uromodulin level was an independent risk factor for rapid eGFR decline in patients with CKD resulting from IgA nephropathy. In addition, the expression level of urinary uromodulin level was associated with interstitial fibrosis as well as tubular atrophy [16]. These studies all supported the viewpoint that either serum/plasma or urinary uromodulin might have great potential for diagnosis of CKD at an early stage and predict its prognosis.

One limitation of our study is the relatively small sample size. Large cohort studies in the future

are needed to conduct to further confirm the clinical values of plasma uromodulin for CKD. In addition, in depth investigation about the molecular mechanisms accounting for the role of uromodulin in regulating the normal kidney function are needed.

Conclusion

Lower expression of plasma uromodulin levels were significantly associated with poorer clinical outcome of CKD, suggesting that plasma uromodulin may be a useful biomarker for predicting the progression of this deadly disease.

Disclosure of conflict of interest

None.

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