doi.org/10.36721/PJPS.2021.34.6.SP.2417-2422.1

Correlation of serum uromodulin levels with renal fibrosis and renal function progression in patients with CKD

Chen Jie, Ying Yi-Ying and Chen Miao*
Department of Nephrology, Ningbo Yinzhou No. 2 Hospital, Ningbo, Zhejiang Province, China

Abstract: To explore the relationship of serum uromodulin levels with renal function progression and renal fibrosis in chronic kidney disease (CKD) patients. Totally 168 CKD patients treated in Department of Nephrology of the present hospital between June 2017 and June 2019 were recruited. These patients were allocated to the deterioration and control groups according to the estimated glomerular filtration rate (eGFR). Multi-factor Logistic regression analysis was utilized for the correlation factors influencing renal function progression and the correlation between serum uromodulin and renal fibrosis was also compared. Number of patients receiving ARB or ACEI drugs intervention was lower in the deterioration group than that in the control group (P<0.05). 24-hour urine protein quantification and HA were higher, while eGFR and uromodulin were lower in the deterioration group compared with the control group (both P<0.05). eGFR (OR=0.373) and uromodulin (OR=0.717) were the protective factors for renal function progression. The risk of renal fibrosis was higher in the deterioration group compared with the control group (P<0.05). Uromodulin was significantly higher in the fibrosis group compared with the normal group (P<0.05). Serum uromodulin is an independent risk factor for renal function progression, and is remarkably correlated with renal fibrosis, which deserves clinical promotion.

Keywords: Chronic kidney disease, estimated glomerular filtration rate, renal fibrosis, serum uromodulin.

INTRODUCTION

Chronic kidney disease (CKD) is defined by functional or structural abnormalities in the kidney for more than 3 consecutive months (Ku *et al*., 2020), and the prevalence in China is about 10.8% (Wang *et al*., 2020). At present, the majority of patients are incurable with high mortality. As the disease progresses, renal fibrosis continuously aggravates and renal function continuously declines, eventually developing into the uremic phase (Lew *et al*., 2018), which cause great physical and mental distress to both patients and their families and impose substantial economic burden. The pathogenesis of renal fibrosis in CKD has been continuously studied, yielding poor progress (Lamprea-Montealegre *et al*., 2020). Initial CKD renal damage is reversible, but renal damage is no longer reversible upon stage 3 (Bajaj *et al*., 2019). The etiotropic treatment is formulated as optimal regimen, which is expected to slow down renal fibrosis and renal function progression, and improve the prognosis. Therefore, early recognition and early intervention are of great significance in CKD and are key topics in clinical studies. Uromodulin, secreted by the renal tubules, is the protein with the highest content in the urine of healthy people (Devuyst *et al*., 2017). Studies have demonstrated that, the quantity of residual intact nephron following renal damage can be evaluated with serum uromodulin, which reflect renal function state (Delgado *et al*., 2017). However, the association between serum uromodulin and CKD is scarcely studied, especially for renal fibrosis and renal function progression. The present study is designed to investigate the correlation of serum uromodulin with renal function progression and renal fibrosis in CKD patients, reported as follows.

MATERIALS AND METHODS

General data

Totally 168 CKD patients at Stages 2 and 3 who were treated in the Department of Nephrology, Ningbo Yinzhou No. 2 Hospital from June 2017 to June 2019 were enrolled in the present study. The present study was approved by the Hospital Ethics Committee after review. Patients and their families signed the informed consent. Inclusion criteria are as follows: (1) patients aged 18-80 years old; (2) those met the diagnostic criteria for CKD in KDIGO (Chang *et al*., 2017) and defined as Stages 2 and 3 according to the staging criteria of US National Kidney Foundation Guideline (Kidney Disease: Improving Global Outcomes (KDIGO), 2013), with eGFR ranging 30-60 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$; (3) patients with complete clinical data and who were able to cooperate throughout the trial. Exclusion criteria: (1) patients with renal tumor requiring surgical treatment or having undergone renal transplantation; (2) those with malignant tumor, severe autoimmune or rheumatic system disease; (3) those accompanied with severe hepatic insufficiency or lung disease.

Methods

All enrolled patients were monitored for blood pressure, heart rate, weight and other items, and were inquired for

**Corresponding author:* e-mail:15888025580@139.com

the current medical history, past history, family history, current treatment and diet. Venous blood was collected in early morning after 8-h fasting. Hematology, kidney function, liver function, serum uromodulin, blood glucose, and blood lipid were tested. Calculated with MDRD formula, eGFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) = 175 × Cr (μmol/L) 1.234 × age (years) - 0.179 and multiplied by 0.79 for women. All patients received disease and health education, and were instructed on daily exercise and diet, and were treated with medication and symptomatic treatment on time.

Follow-up

All patients were followed up in outpatient clinic visits (or by telephone) for 12 months, once per month. Patients were inquired for the detailed disease condition and instructed on medication. Urea nitrogen (BUN) and serum creatinine (SCr) were detected every 6 months, and eGFR were measured. Renal biopsy was conducted to detect renal fibrosis at the end of follow-up.

Outcome measures

(1) General data: including age, gender, heart rate, blood pressure, co-morbidities, family history. (2) Laboratory indexes: including blood platelet count, albumin, triglyceride (TG), fasting blood glucose (GLU), total cholesterol (TC), haemoglobin (HGB), alanine aminotransferase (ALT), serum uromodulin. (3) Renal function Progression in CKD (Kidney Disease: Improving Global Outcomes (KDIGO), 2013): the decline of eGFR ≥ 5 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$ within 1 year or CKD developed to Stage 5 (or requiring renal replacement therapy). (4) Patients' renal fibrosis: the occurrence of renal fibrosis in patients was judged according to renal biopsy results (figs. 1, 2, 3 and 4).

STATISTICAL ANALYSIS

All data were processed using SPSS 20.0 statistical software. Count data were analyzed using χ^2 test for inter-group comparison. Measurement data were presented as ($\bar{x}\pm s$). Independent-sample *t*-test was utilized for inter-group comparison. In multi-factor Logistic regression analysis, the forward algorithm method was applied to screen independent variables and the statistically significant factors were involved in the analysis. The influence of relevant factors on CKD renal function progression was analyzed and the correlation between serum uromodulin and renal fibrosis was compared. A level at $P<0.05$ was considered statistically significant.

RESULTS

Follow-up

Totally 168 CKD patients were finally enrolled in the present study. Nine cases were lost during 12-month follow-up and managed as drop-out cases. The remaining 159 patients were allocated to the deterioration group (n=43, presence of renal function progression) and the control group (n=116). The incidence of renal function progression was 27.04% (43/159) in CKD patients. Renal fibrosis occurred in 22 of the 159 CKD patients, classified as the fibrosis group, while the remaining 137 patients were classified as the normal group. The incidence of renal fibrosis was 13.84% (22/159). Renal fibrosis was visible in the histological slides in the deterioration group.

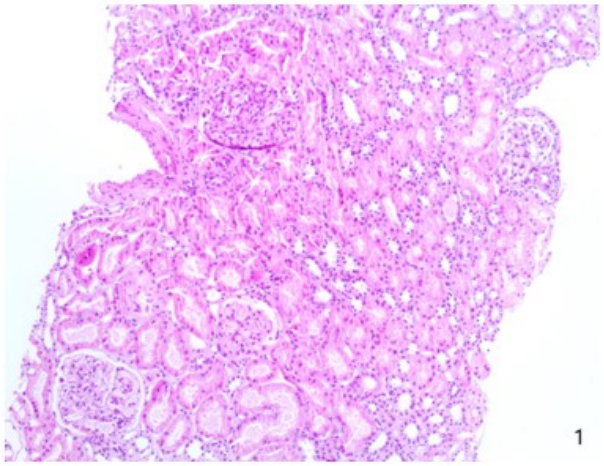

Fig. 1: HE staining of kidney tissue.

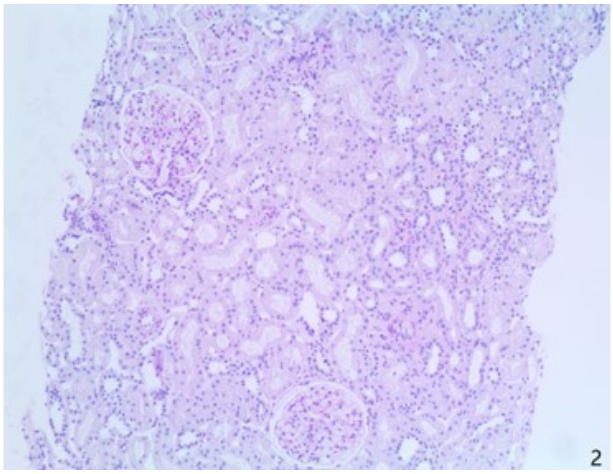

Fig. 2: PAS staining of kidney tissue.

Comparison of general data between the deterioration and control groups

The statistical difference was not significant in the general data such as gender and age between the deterioration and control groups ($P>0.05$); however, the number of patients receiving ARB or ACEI drugs intervention was lower in the deterioration group compared with the control group ($P<0.05$) (table 1).

Comparison of laboratory indexes between the deterioration and control groups

The statistical difference was not significant in the laboratory indexes such as GLU and UA between the two groups ($P>0.05$). However, 24-hour urine protein

quantification and HA were higher in the deterioration group compared with the control group (P<0.05), while eGFR and uromodulin were lower in the deterioration group compared with the control group (P<0.05) (table 2).

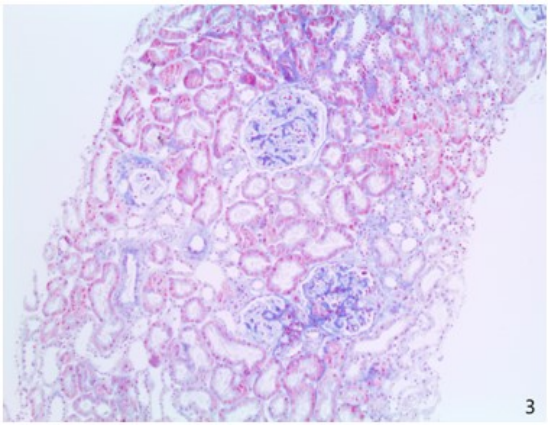

Fig. 3: Masson staining of kidney tissue. (10x10).

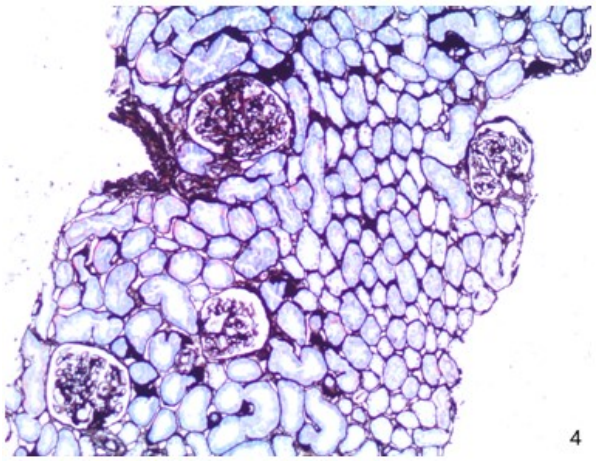

Fig. 4: PAS+ Masson staining of kidney tissue

Multi-factor Logistic regression analysis affecting renal function progression in CKD patients

Multi-factor Logistic regression analysis showed that: eGFR (OR=0.373) and uromodulin (OR=0.717) were the protective factors for renal function progression in CKD patients (table 3).

Comparison of renal fibrosis between the two groups

The risk of renal fibrosis was increased in the deterioration group compared with the control group (P<0.05) (table 4).

Comparison of the risk of renal fibrosis in CKD patients

Uromodulin was significantly increased in the fibrosis group compared with the normal group (P<0.05) (table 5).

DISCUSSION

In recent years, due to the aging of the population and the increased prevalence of metabolic diseases, the incidence rate of CKD is yearly increasing in China (Ferro *et al*., 2018). Renal function in patients gradually deteriorates and progresses to end stage renal disease (ESRD) with the progression of CKD disease, which is manifested as renal fibrosis (Chinese Nephrologist Association and The Working Group of Chinese Practice Program of Vitamin D, 2020). Therefore, early recognition and intervention of CKD by using various endogenous markers of renal damage is of great significance to prevent the progression of CKD to ESRD.

Uromodulin is a glycoprotein consisting of 640 amino acids with a molecular weight of 95 kD, is synthesized by the endoplasmic reticulum in epithelial cells of the ascending branch of renal tubular medullary loops, and it is also known as Tamm-Horsfall glycoprotein or uromucoid (Tokonami *et al*., 2018). It is released into the urine after hydrolytic cleavage by proteases in epithelial cells. It has antimicrobial effects and prevents urinary tract infections; it also regulates the activity of renal ion channels, balances osmotic pressure and regulates intertubular communication (Garimella *et al*., 2017). Evidence from a study (Tokonami *et al*., 2018) supports that, serum uromodulin is subnormal at initial stage of renal function impairment, even though the SCR and BUN levels of patients are still within the normal range, thus it is proposed that serum uromodulin could be used as a predictor of impaired renal function in early CKD. However, studies on its association with renal function progression and renal fibrosis are few.

In this study, 43 of the enrolled 168 CKD patients experience renal function progression, and the incidence of renal function progression in CKD patients is 27.04%. There is no significant difference regarding general data such as gender and age between the deterioration and control groups. However, the number of patients in the deterioration group treated with ARB or ACEI drug intervention is lower compared with the control group. This finding has confirmed that the application of ARB or ACEI drug intervention can delay renal function progression and have a protective effect against the deterioration of CKD patients.

The effect is mainly related to the fact that ARB or ACEI drugs inhibit the renin-angiotensin-aldosterone system activation, decrease the glomerular pressure and blood pressure and reduce the excretion of urinary protein, thus effectively delay renal disease progression and have a protective effect on renal function.

There is no significant difference regarding laboratory indexes such as GLU and UA between the two groups; however, 24-hour urine protein quantification and HA are increased in the deterioration group compared with the control group, while eGFR and uromodulin are lower in the deterioration group than in the control group. 24-hour urine protein quantification and eGFR are important indexes for assessing renal function impairment.

Table 1: Comparison of general data between the deterioration and control groups [(x ± s), *n*(%)]

Item		Deterioration group (*n*=43)	Control group (*n*=116)	t/χ^2	*P*
Age (year)		56.34±10.41	55.25±11.19	0.556	0.579
CKD family history (*n*)		4(9.30)	11(9.48)	0.001	0.972
Gender (*n*)	Female	20(46.51)	55(47.41)	0.010	0.919
	Male	23(53.49)	61(52.89)		
Systolic pressure (mmHg)		119.72±11.54	121.03±10.68	0.672	0.502
Diastolic pressure (mmHg)		70.85±9.87	71.59±9.01	0.448	0.655
With hyperlipidemia (*n*)		7(16.28)	18(15.52)	0.014	0.907
With coronary heart disease (*n*)		13(30.23)	38(32.76)	0.092	0.762
With diabetes mellitus (*n*)		5(11.63)	16(13.79)	0.128	0.720
With hypertension (*n*)		12(27.91)	22(18.97)	1.492	0.222
alcohol drinking history (*n*)		6(13.95)	24(20.69)	0.930	0.335
Smoking history (*n*)		7(16.28)	23(19.83)	0.258	0.611
Weight (kg)		70.16±9.48	68.77±10.15	0.780	0.436
Intervention (*n*)	Diet and exercise intervention	41(95.35)	114(98.28)	1.096	0.295
	ARB or ACEI drug intervention	28(65.12)	96(82.76)	5.688	0.017
	Other drug intervention	35(81.40)	104(89.66)	1.946	0.163

Table 2: Comparison of laboratory indexes between the deterioration and control groups (x ± s)

Item	Deterioration group (*n*=43)	Control group (*n*=116)	*t*	*P*
GLU (mmol/L)	4.79±1.15	5.12±1.07	1.693	0.093
TG (mmol/L)	2.07±0.52	1.92±0.53	1.593	0.113
UA (umol/L)	275.64±20.71	280.53±21.02	1.308	0.193
TC (mmol/L)	5.78±1.25	5.57±1.18	0.981	0.328
ALT (U/L)	8.46±2.32	8.94±2.29	1.170	0.244
Blood platelet count ($\times10^9$/L)	146.61±40.27	143.82±39.86	0.391	0.696
HGB (g/L)	109.88±10.47	111.16±9.86	0.715	0.476
BUN (mIU/L)	12.74±3.12	13.83±3.25	1.898	0.059
SCr (ng/ml)	103.74±11.45	106.39±10.53	1.376	0.171
24-hour urine protein quantification (g)	1.90±0.45	1.29±0.41	8.114	0.000
Albumin (g/L)	37.37±4.24	48.85±4.38	1.909	0.058
HA (ug/L)	88.56±25.41	69.55±20.84	4.806	0.000
eGFR (ml•min^{-1}•1.73 m^{-2})	31.13±4.29	37.48±5.36	6.980	0.000
Uromodulin (mg/mL)	45.58±9.65	64.06±10.44	10.113	0.000

Table 3: Multi-factor Logistic regression analysis affecting renal function progression in CKD patients

Factor	β	Standard error	Wald	*P*	OR	95% confidence interval	
						Lower limit	Upper limit
ARB or ACEI drug intervention	-2.504	1.398	3.207	0.073	0.082	0.005	1.267
24-hour urine protein quantification	2.646	1.402	3.562	0.059	14.097	0.903	220.036
HA	0.023	0.019	1.414	0.234	1.023	0.985	1.063
eGFR	-0.985	0.355	7.681	0.006	0.373	0.186	0.749
Uromodulin	-0.332	0.094	12.554	0.000	0.717	0.597	0.862

Table 4: Comparison of renal fibrosis between the deterioration and control groups [*n*,%]

Group	*n*	With	Without	Total incidence
Deterioration	43	21(48.84)	22(51.16)	12(26.66)
Control	116	1(0.86)	115(99.14)	13(10.40)
χ^2				60.564
P				0.000

Table 5: Comparison of the risk of renal fibrosis in CKD patients (x±s)

Item	Fibrosis group (*n*=22)	Normal group (*n*=137)	*t*	*P*
Uromodulin (mg/mL)	40.05±7.96	62.12±10.59	9.350	0.000

Among them, 24-hour urine protein quantification is a risk factor for decreased renal function, and plays an important role on guiding CKD treatment, evaluating treatment effect and judging prognosis, mainly reflecting glomerular filtration and tubular reabsorption impairment (Leiherer *et al*., 2018). eGFR is a crucial functional index for CKD staging and diagnosis and mainly indicates glomerular filtration rate. The lower eGFR indicates the higher staging and worse renal function of CKD patients. HA has a predictive effect on renal fibrosis and can indirectly reflect the progression of renal function. The higher HA level indicates the more serious renal function decline and renal fibrosis, as well as the faster progression of the disease (Bostom *et al*., 2018). Serum uromodulin is mainly related to the quantity of intact nephron and is currently applied to assess early renal damage. The lower uromodulin level indicates the higher quantity of damaged nephron and the faster progression of renal function. The results of a complete genome-wide study (Garimella *et al*., 2017) have shown that, polymorphisms in genes on uromodulin mononucleotides are significantly correlated with the progression and occurrence of CKD. Uromodulin is critical for the evaluation of renal function.

Multi-factor Logistic regression analysis demonstrates that: uromodulin and eGFR are protective factors for renal function progression in CKD patients. 24-hour urine protein quantification level is proportional to renal function progression, and the more serious renal injury is indicative of the more protein leaked from the kidney, the lower eGFR and the worse renal function of the patients. In contrast, serum uromodulin directly reflects the renal function. The faster progression of renal function is indicative of the more serious renal damage and the less uromodulin secreted by its endothelial cells, proving the protective effect of uromodulin on the kidney.

In this study, renal fibrosis occurs in 22 of the remaining 159 CKD patients, and the incidence of renal fibrosis is 13.84%. The risk of renal fibrosis is increased in the deterioration group compared with the control group. The uromodulin level is significantly increased in the fibrosis group compared with the normal group. This finding indicates that the faster progression of renal function of CKD patients correlates with the higher grading and the higher incidence of renal fibrosis. The occurrence of renal fibrosis is inversely proportional to serum uromodulin, the lower uromodulin level indicates the lower incidence of renal fibrosis. Renal fibrosis occurs at the end stage of CKD, with renal failure and obvious damage to renal cells, while renal fibrosis is characterized by a decreasing number of innate cells in renal glomerulus, excessive deposition of extracellular matrix, proliferation of renal interstitial fibroblasts and loss of interstitial capillaries, which are directly related to the damage of renal cells. Once renal epithelial cells are damaged, the secreted uromodulin is significantly decreased. The serious renal fibrosis induces the lower serum uromodulin level, which are inversely proportional and obviously correlated. However, due to insufficient number of patients included in the present study, long-term studies with large samples are required.

CONCLUSION

In conclusion, serum uromodulin, an independent risk factor for renal function progression, has a significant correlation with renal fibrosis in CKD patients, which deserve clinical promotion.

REFERENCES

Bajaj A, Xie D, Cedillo-Couvert E, Charleston J, Chen J, Deo R, Feldman HI, Go AS, He J, Horwitz E, Kallem R, Rahman M, Weir MR, Anderson AH, Rader DJ and CRIC Study Investigators (2019). Lipids, apolipoproteins and risk of atherosclerotic cardiovascular disease in persons with CKD. *Am. J. Kidney Dis*., **73**(6): 827-836.

Bostom A, Steubl D, Garimella PS, Franceschini N, Roberts MB, Pasch A, Ix JH, Tuttle KR, Ivanova A, Shireman T, Kim SJ, Gohh R, Weiner DE, Levey AS, Hsu CY, Kusek JW and Eaton CB (2018). Serum uromodulin: a biomarker of long-term kidney allograft failure. *Am. J. Nephrol*., **47**(4): 275-282.

Chang CC, Chen CY, Huang CH, Wu CL, Wu HM, Chiu PF, Kor CT, Chen TH, Chang GD, Kuo CC, Wen HC, Huang CY and Chang CH (2017). Urinary glycated uromodulin in diabetic kidney disease. *Clin. Sci. (Lond).*, **131**(15): 1815-1829.

Chinese Nephrologist Association, The Working Group of Chinese Practice Program of Vitamin D (2020). The application of vitamin D and its analogues in patients with chronic kidney disease: The Chinese practice program (2019). *Zhonghua Neike Zazhi*, **59**(02): 104-116.

Delgado GE, Kleber ME, Scharnagl H, Kramer BK, März W and Scherberich JE (2017). Serum uromodulin and mortality risk in patients undergoing coronary angiography. *J. Am. Soc. Nephrol*., **28**(7): 2201-2210.

Devuyst O, Olinger E and Rampoldi L (2017). Uromodulin: From physiology to rare and complex kidney disorders. *Nat. Rev. Nephrol*., **13**(9): 525-544.

Ferro CJ, Mark PB, Kanbay M, Sarafidis P, Heine GH, Rossignol P, Massy ZA, Mallamaci F, Valdivielso JM, Malyszko J, Verhaar MC, Ekart R, Vanholder R, London G, Ortiz A and Zoccali C (2018). Lipid management in patients with chronic kidney disease. *Nat. Rev. Nephrol.,* **14**(12): 727-749.

Garimella PS, Bartz TM, Ix JH, Chonchol M, Shlipak MG, Devarajan P, Bennett MR and Sarnak MJ (2017). Urinary uromodulin and risk of urinary tract infections: The cardiovascular health study. *Am. J. Kidney Dis*., **69**(6): 744-751.

Garimella PS and Sarnak MJ (2017). Uromodulin in

kidney health and disease. *Curr. Opin. Nephrol. Hypertens.,* **26**(2): 136-142.

Kidney Disease: Improving Global Outcomes (KDIGO). (2013). KDIGO 2012 clinical practice guideline for evaluation and management of chronic kidney disease. *Kidney Int. Suppl*., **3**(1): 1-150.

Ku E, Yang W, McCulloch CE, Feldman HI, Go AS, Lash J, Bansal N, He J, Horwitz E, Ricardo AC, Shafi T, Sondheimer J, Townsend RR, Waikar SS, Hsu CY and CRIC Study Investigators (2020). Race and mortality in CKD and dialysis: Findings from the chronic renal insufficiency cohort (CRIC) study. *Am. J. Kidney Dis.*, **75**(3): 394-403.

Lamprea-Montealegre JA, Staplin N, Herrington WG, Haynes R, Emberson J, Baigent C, de Boer IH and SHARP Collaborative Group (2020). Apolipoprotein B, triglyceride-rich lipoproteins, and risk of cardio-vascular events in persons with CKD. *Clin. J. Am. Soc. Nephrol*., **15**(1): 47-60.

Leiherer A, Muendlein A, Saely CH, Brandtner EM, Geiger K, Fraunberger P and Drexel H (2018). The value of uromodulin as a new serum marker to predict decline in renal function. *J. Hypertens*., **36**(1): 110-118.

Lew QLJ, Allen JC, Nguyen F, Tan NC and Jafar TH (2018). Factors associated with chronic kidney disease and their clinical utility in primary care clinics in a multi-ethnic Southeast Asian population. *Nephron*, **138**(3): 202-213.

Tokonami N, Olinger E, Debaix H, Houillier P and Devuyst O (2018). The excretion of uromodulin is modulated by the calcium-sensing receptor. *Kidney Int*., **94**(5): 882-886.

Tokonami N, Takata T, Beyeler J, Ehrbar I, Yoshifuji A, Christensen EI, Loffing J, Devuyst O, Olinger EG (2018). Uromodulin is expressed in the distal convoluted tubule, where it is critical for regulation of the sodium chloride cotransporter NCC. *Kidney Int*., **94**(4): 701-715.

Wang M, Zhang X, Sui Z, Bai L, Wang Y, Zuo L and Wang M (2020). Impact of arterial stiffness on prognosis in non-diabetic pre-dialysis patients with stages 3-5 chronic kidney disease. *Zhonghua Linchuang Yishi Zazhi (Electronic Edition)*, **14**(03): 170-174.