ROLE OF SERUM UROMODULIN IN THE EARLY DIAGNOSIS OF CHRONIC KIDNEY DISEASE

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Abstract. Background: The diagnosis of chronic kidney disease (CKD) is usually delayed, when significant functional renal impairment has already occurred. The diagnosis is complex and clinical and laboratory investigations play a crucial role. There are well-established markers of CKD – serum creatinine and cystatin C. However, the search for new reliable biomarkers that aid in the assessment of kidney function and predict the evolution of the disease is still in progress. **Objective:** To investigate the role of serum uromodulin (sUmod) as a marker for early diagnosis of renal impairment in patients with CKD. **Materials and Methods:** We investigated 70 patients, 28 male and 42 female, mean age 56.53 ± 11.753 , with CKD in a prospective observational study. All patients were admitted to the Clinic of Nephrology at the "St. Ivan Rilski" University Hospital between April and November 2019. After obtaining written informed consent, laboratory blood and urine tests, abdominal ultrasound and sUmod investigations were performed in all patients. **Results:** Plasma uromodulin levels showed decrease with the increasing of the severity of renal impairment. sUmod displayed inverse correlation with serum creatinine ($r = -0.467$, $p < 0.0001$), cystatin C ($r = -0.430$, $p < 0.0001$) and urea ($r = -0.495$, $p < 0.0001$) and a positive correlation with eGFR ($r = 0.628$, $p < 0.0001$). **Conclusion:** The results of our study show that sUmod levels significantly correlate with all established laboratory parameters used for the evaluation of renal impairment. It can be used as a potential early biomarker for CKD diagnosis.

Key words: serum uromodulin, chronic kidney disease, biomarker, serum creatinine, cystatin C

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INTRODUCTION

The diagnosis of chronic kidney disease (CKD) is usually belated, when significant functional renal impairment has already occurred, due to its nonspecific symptoms. CKD encompasses five stages, and progresses slowly from mild to moderate

and to severe reduction in glomerular filtration. The risk for the development of CKD increases with age. The diagnosis is complex and is based on laboratory and instrumental tests and kidney biopsy [1-5].

The clinical-laboratory investigations have crucial role for the diagnosis of CKD, because they provide fast, easy, and relatively inexpensive tests with

discriminative results. Although there are well-established markers, such as serum creatinine (SCr) and cystatin C (CysC), the search for new reliable biomarkers that would aid the assessment of kidney function and the prediction of the evolution of CKD is still in progress [6-7]. Several new markers for the evaluation of renal function recently have been introduced for scientific purposes, and some of them are being used routinely. Uromodulin, also known as Tamm-Horsfall protein, is a promising marker for the diagnosis of renal damage, but its clinical significance needs more scientific evaluation [8-11]. Uromodulin is produced almost entirely by the kidney and is the most abundant protein excreted with the normal urine. In the past, urinary uromodulin has been studied more extensively, but the role of serum uromodulin (sUmod) remains unclear. sUmod is a stable monomeric antigen and it seems a more reliable marker than urinary [12, 13].

The aim of the study was to investigate the role of sUmod as a marker for early diagnosis of renal impairment in patients with chronic renal diseases.

MATERIAL AND METHODS

Overall 70 patients (28 male and 42 female, mean age 56.53 ± 11.753) diagnosed with CKD were included in this prospective observational study. All subjects were admitted to the Clinic of Nephrology at the University Hospital St. Ivan Rilski" between April and November 2019. The study was approved by the local ethics committee of Medical University, Sofia, Bulgaria, in adherence with the declaration of Helsinki. Prior to the inclusion in the study, written informed consent was obtained from all studied subjects. All the patients were > 18 years of age. Arterial hypertension had 64/70 (91.43%) and 39/70 (55.71%) had diabetes, with optimal glycaemic control at the time of the investigation (in the cases with diabetes) defined as HbA1c < 7%. Patients with acute or chronic infections, mental disorders, organ transplant recipients, proven oncological diseases, prolonged use (over the last six months) and during the study of non-steroidal anti-inflammatory drugs, corticosteroids, hormonal preparations or antioxidants were excluded from the study. Furthermore, patients with symptomatic or asymptomatic urinary tract infection, defined as detection of leucocytes and/or bacteria in the urinary sediment at the time of enrollment, were excluded.

Medical history and physical examination were performed in all participants. Haematological and biochemical blood tests and urine tests were performed and 24-hours proteinuria was calculated, using standard methods. Estimated glomerular filtration rate (eGFR)

was evaluated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation: $eGFR (mL/min/1.73 m^2) = 175 \times SCr^{-1.154} \times Age^{-0.203} \times 0.742$ (if woman) $\times 1.21$ (if black) [14,15].

CysC was measured using Human ELISA Cystatin C kit (Biovendor Research and Diagnostic Products, Cat. No: RD191009100, Germany) by the particle enhanced immune nephelometric method (normal range 0.50-0.96 mg/L). In all patients abdominal ultrasound with duplex Doppler examination of the kidneys was performed.

The investigation of sUmod was performed with uromodulin-ELISA kit (Euroimmun AG, Lübeck, Germany). Overall 5 mL venous blood was collected from each participant. Then the plasma was prepared by centrifugation at 2000×g for 30 min at 4°C. All plasma samples were stored at – 80°C before measurements were performed. A 96-well plate was coated with uromodulin in advance and blocked to reduce non-specific binding. Plasma samples were diluted 1:101 using dilution buffer. A total of 100 µL of calibrators, controls, or diluted samples were pipetted into coated wells of the microtiter plate, subsequently 100 µL of biotinylated detection antibody (final concentration 50 ng/mL) were added. The microtiter plate was covered with foil and incubated for 120 minutes at 450 rotations per minute (rpm) and room temperature (+18°C to +25°C) on a rotary shaker. After 2 hours the microtiter plate was washed 3 times using 300µL washing buffer, then the wells were tapped gently. A total of 100 µL of streptavidin-peroxidase (final concentration 67ng/mL) were pipetted into each well followed by another incubation for 30 minutes at 450 rpm. Subsequently, the streptavidin-peroxidase was soaked and the microtiter plate washed 3 times with 300 µL of washing buffer. Consequently, 100 µL of substrate solution (containing the chromogen tetramethylbenzidine and hydrogen peroxide as the substrate for streptavidin-peroxidase) were pipetted into each well. The microtiter plate was incubated in the dark for 15 minutes at room temperature. The reaction was terminated by adding 100 µL of stop solution. This causes a color change from blue to yellow. Finally, the substrate solution was measured using a photometer at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm. For this assay the mean linearity recovery was 97% (83%-107% at 59-397 ng/mL); intra-assay precision –1.8-3.2% (at 30-214 ng/mL), inter-assay precision – 6.6% to 7.8% (at 35-228 ng/mL), and inter-lot precision – 7.2% to 10.1% (at 37-227 ng/mL). The lower detection limit of the uromodulin ELISA was 2.0 ng/mL.

The **statistical analysis** was performed using SPSS vs.19 for Windows. The statistical analyses included a variational analysis of the quantitative

variables – median, standard deviation, standard error of the median and 95% confidence interval of the median and frequency analysis of qualitative variables. Kolmogorov-Smirnov, Mann-Whitney and Kruskal-Wallis Tests were used for testing the normality of distribution of the quantitative variables. Correlations were tested using the Spearman's tests. All data are presented as mean values $\pm$ SD or as numbers, unless specified otherwise. All values of $P \leq 0.05$ were considered statistically significant.

RESULTS

The study included 70 patients with chronic renal impairment, 28 male and 42 female, mean age of the patients was 56.53 ± 11.753 years. The male-to-female ratio was 28/42 (40.0% men and 60.0% women). The main comorbidities were arterial hypertension – in 64 (91.43%) and diabetes mellitus – in 39 (55.71%). The main clinical and laboratory data of patients are presented in Table 1.

Table 1. Characteristics of the studied patients

Parameter	Mean $\pm$ SD
Weight (kg)	79.99 $\pm$ 19.119
Height (cm)	167.51 $\pm$ 8.893
Body Mass Index	28.354 $\pm$ 5.844
Systolic blood pressure (mm Hg)	136.79 $\pm$ 15.972
Diastolic blood pressure (mm Hg)	87.07 $\pm$ 10.197
Uric acid (μ mol/L)	383.06 $\pm$ 112.256
SCr (μ mol/L)	191.17 $\pm$ 156.538
Urea (mmol/L)	12.136 $\pm$ 7.669
eGFR (mL/min/1.73 m ²)	40.83 $\pm$ 26.019
CysC (mg/L)	2.549 $\pm$ 1.636
sUmod (ng/ml)	123.237 $\pm$ 128.611
SCr = serum creatinine, eGFR = estimated glomerular filtration rate (MDRD), CysC = Cystatin C, sUmod = serum uromodulin.	

The patients were divided into 5 groups according to the KDIGO Recommendations: CKD stage I (eGFR > 90 mL/min/1.73 m²) – 11; CKD stage II (eGFR: 60 to 89 mL/min/1.73 m²) – 17; stage IIIa (eGFR: 45 to 59 mL/min/1.73 m²) – 6; CKD stage IIIb (eGFR: 30 to 44 mL/min/1.73 m²) – 13; CKD stage IV (eGFR: 15 to 29 mL/min/1.73 m²) – 13; CKD stage V (eGFR < 15 mL/min/1.73 m²) – 10. The mean levels of sUmod, CysC, SCr and urea in the different groups are presented in Table 2.

The correlation analysis revealed a negative correlation between sUmod and SCr ($r = [-0.467]$, $p < 0.0001$), CysC ($r = [-0.430]$, $p < 0.0001$) and urea ($r = [-0.495]$, $p < 0.0001$), and a positive relation with eGFR ($r = 0.628$, $p < 0.0001$).

DISCUSSION

SCr and urea levels, CysC and eGFR are well-established markers for the diagnosis of CKD. Yet, these markers seem to be insufficient and sometimes inaccurate for early diagnosis [9]. Therefore, new validated biomarkers are needed for a better assessment of CKD and its progression. We investigate the role of sUmod as a marker for early diagnosis of renal impairment in CKD. In our study the mean plasma uromodulin level was 123.2367 ng/mL for all CKD stages combined (from 2.64 to 709.64 ng/mL) and sUmod decreased with the increasing of the degree of renal impairment. The lower values were measured in higher stages of CKD. Similar results were obtained by Scherberich et al. [16], who reported similar sUmod levels – from 1 to 667 ng/mL with a median of 85 ng/mL. In their study sUmod decreased with progressive CKD as well [16].

Previous studies found that sUmod concentrations were significantly lower among patients with CKD than in controls and displayed inverse correlation with creatinine [17-19]. Our results confirm once

Table 2. Mean levels of sUmod, CysC, serum creatinine and urea in different CKD groups

CKD stage	sUmod (ng/ml)	CysC (mg/L)	SCr (μ mol/L)	Urea (mmol/L)
I (n = 11)	228.008 $\pm$ 176.267	1134.337 $\pm$ 384.683	74.00 $\pm$ 11.636	4.862 $\pm$ 1.809
II (n = 17)	200.889 $\pm$ 117.161	1502.398 $\pm$ 759.831	78.41 $\pm$ 9.428	5.297 $\pm$ 1.244
IIIa (n = 6)	109.015 $\pm$ 48.434	2165.468 $\pm$ 758.963	118.33 $\pm$ 18.118	9.100 $\pm$ 4.619
IIIb (n = 13)	74.464 $\pm$ 50.127	3153.244 $\pm$ 2028.953	157.85 $\pm$ 33.148	12.070 $\pm$ 2.364
IV (n = 13)	45.112 $\pm$ 20.547	3384.859 $\pm$ 965.872	264.62 $\pm$ 50.791	20.54 $\pm$ 5.043
V (n = 10)	42.439 $\pm$ 35.050	4129.085 $\pm$ 1812.850	503.30 $\pm$ 143.146	24.870 $\pm$ 6.666
sUMOD = serum uromodulin; CysC = cystatin C; SCr = serum creatinine; all data are presented as mean values $\pm$ SD.				

again that plasma uromodulin expression significantly negatively correlates with SCr levels ($r = [-0.467]$).

Steubl et al. tested plasma uromodulin in 426 individuals of whom 71 were CKD stage 0 and 355 had CKD and found that uromodulin correlated well with CysC ($r = [-0.79]$) [19]. Similar results were reported by other authors [16, 18]. In our study sUmod concentrations are strongly negatively correlated with renal CysC ($r = [-0.353]$).

Risch et al. [18] tested 289 participants with different stages of kidney function (i.e., cystatin C-based 2012-CKD-EPI eGFR CysC > 90 mL/min/1.73 m², 60-89 mL/min/1.73 m², 45-59 mL/min/1.73 m², and < 45 mL/min/1.73 m²). They found significant differences in serum uromodulin among the four groups according to different kidney function stages ($p < 0.001$). sUmod displayed inverse relationships with urea ($r = -0.30$) [18]. Scherberich et al. [16] and Steubl et al. [19] also found significant negative correlation between sUmod and BUN ($r = -0.645$ and -0.72 respectively). Our results are similar and sUmod concentrations show strong negative correlation with urea ($r = [-0.495]$).

Lv et al. investigated 2652 CKD patients and found a positive correlation between sUmod and eGFR in multivariable linear correlation analysis ($r = 0.68$, $p < 0.001$) [10]. Similar significant relation have been reported by Steubl et al. [19] and Leiherer et al. [20] ($r = 0.80$ and $r = 0.242$ respectively). Our results show statistically significant correlation between sUmod and eGFR ($r = 0.628$).

STUDY LIMITATIONS

One limitation of our study is the relatively small sample size and the single-center recruitment. The number of patients in the different CKD groups is not sufficient for in-depth statistical analysis. Large cohort studies in the future are needed to confirm our findings.

CONCLUSIONS

Our study shows that sUmod levels significantly correlate with all well-known and already established laboratory parameters used for evaluation of renal impairment. Our findings support the use of sUmod as a potential early biomarker for the diagnosis of CKD.

Disclosure summary: *The authors have nothing to disclose.*

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