SERUM UROMODULIN - A MARKER FOR DIAGNOSIS OF CHRONIC KIDNEY DISEASES

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ABSTRACT

Introduction*: Chronic kidney disease is one of the most frequent chronic diseases causing disability and a significant decrease in quality of life. A major role in its diagnosis plays the clinical laboratory because it provides fast, easy and relatively cheap methods. Although there are well-established markers such as serum creatinine and cystatin C, the search for new reliable biomarkers to help assess kidney function and to predict the evolution of the disease continues. One of them can be Uromodulin, also known as Tamm-Horsfall protein. However, its exact function still needs to be clarified.*

The aim of the study was to evaluate the role of serum uromodulin as a marker of the renal impairment in patients with chronic renal diseases.

Materials and methods*: A total of 68 patients were enrolled in this prospective observational study in the Clinic of Nephrology of the University Hospital "St. Ivan Rilski " for a period of two years (2017-2018). The mean age of the patients was 62.21±11.869 years with the male/female ratio 31/37 (45.6% / 54.4%). Laboratory blood and urine tests, abdominal ultrasound with resistive index measurement and serum uromodulin investigations were performed in all patients.*

Results*: Serum uromodulin levels were significantly negatively correlated with serum creatinine (r = -0.720, p <0.0001), urea (r = -0.717, p <0.0001), uric acid (r = -0.296, p = 0.017), cystatin C (r = -0.353, p = 0.004) and resistive index (r = -0.353, p = 0.004). Correspondingly, a positive relationship with estimated glomerular filtration rate (r = 0.692, p <0.0001) was found.*

Conclusion*: Serum uromodulin levels significantly correlate with the resistive index and all already established laboratory parameters used for evaluation of renal impairment. It can be used as a potential marker for diagnosis and early assessment of chronic kidney disease progression.*

Keywords: *serum uromodulin, chronic kidney disease, biomarker, cystatin C, resistive index.*

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Introduction

Chronic kidney disease (CKD) is one of the most frequent chronic diseases causing disability and a significant decrease in quality of life. It occurs all over the world and is associated with an increased mortality risk[1-3]. Because of non-specific symptoms, CKD is diagnosed late when significant functional kidney disorders occur. Diagnosis of CKD is complex, including not only clinical picture, laboratory and instrumental methods but also histological verification by puncture renal biopsy.

The increased level of intrarenal resistive index (RI) in Doppler sonography is associated with CKD and is commonly used for evaluation and prediction of renal diseases[4, 5]. On the other hand, renal biopsy is an essential tool for exact diagnosis, although it is an invasive test with several complications[6, 7].

A major role in the diagnosis of CKD is played by the clinical laboratory as it provides fast, easy and relatively cheap methods[4,5]. Although there are well-established markers such as serum creatinine (SCr) and cystatin C (CysC), the search for new reliable biomarkers to help assess kidney function and to predict the evolution of the disease continues[8-10].

Uromodulin, also known as Tamm-Horsfall protein, is exclusively produced by the kidney and is the most abundant protein excreted in normal urine. According to some publications, it is a promising marker for the diagnosis of renal damage, although its significance has yet to be fully clarified[11-13]. Previous studies mainly focused on urinary uromodulin excretion and its change in case of CKD. On the other hand, serum uromodulin (sUmod) has not been investigated widely yet[14-16].

Furthermore, sUmod is a stable monomeric antigen and measurements performed on it seem to be more reliable[17].

The aim of the study was to evaluate the role of sUmod as a marker of the renalimpairment in patients with chronic renal diseases.

Material and methods

Material

A total of 68 patients diagnosed with CKD were included in this prospective observational study. They were admitted in Clinic of nephrology, UMHAT "St. Ivan Rilski", for a period of 2 years (2017-2018). All patients gave their written informed consent to participate. The protocol was conformed to the guidelines of the 1975 Helsinki Declaration. All the patients were at least 18 years of age with optimal level of glycemic control (HbA1c < 7%) in case of diabetes mellitus. Patients with mental disturbances or who have had organ transplantation, proven oncological diseases, acute or chronic infections, 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.

Methods

The following patient data were assessed: age, gender, body weight, systolic/diastolic blood pressure, history of diabetes mellitus, hypertension or other concomitant diseases. Hematological and biochemical blood tests were performed: complete blood count with differential leukocyte count, SCr, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), potassium, sodium, chlorides, blood glucose and lipid profile. 24-hour proteinuria was calculated. Estimated glomerular filtration rate (eGFR) was evaluated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation: eGFR (mL/min/1.73 m2) = 175 × SCr -1.154 × Age -0.203 × 0.742 (if woman) × 1.21 (if black)[18,19]. CysC was measured with 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).

All patients underwent abdominal ultrasound with duplex Doppler examination of the kidneys. RI was measured at inter-lobar arteries in upper, middle and lower areas of each kidney. The mean value of RI was calculated by deriving it from 6 measurements for each patient. RI was calculated as: (peak systolic velocity - end diastolic velocity)/peak systolic velocity. Values of RI higher than 0.70 were considered pathological[20].

Analysis of sUmod was performed with uromodulin-ELISA kit (Euroimmun AG, Lübeck, Germany). Up to 5 mL of 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 100µL of calibrators, controls, or diluted samples were pipetted into coated wells of the microtiter plate, subsequently 100µL of biotinylated detection antibody (final concentration 50ng/mL) was 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 of washing buffer, then the wells were tapped gently. A total of 100µL of streptavidin-peroxidase (final concentration 67ng/mL) were pipetted into each well followed by another incubation for 30 minutes at 450rpm. Subsequently, the streptavidin-peroxidase was soaked and the microtiter plate was washed 3 times with 300µL of washing buffer. Consequently, 100µL of substrate solution (containing the chromogen tetramethylbenzidin 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 100µL 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 450nm and a reference wavelength between 620nm 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 2.0ng/mL.

Statistical Analysis

The statistical data processing was carried out using SPSS 19.0 statistical software (IBM Software Group, Chicago, USA). The adopted level of significance, eliminating the null hypothesis, was $p<0.05$. The statistical analyses included a variational analysis of the quantitative variables -median, standard deviation, standard error and 95% confidence interval of the median. Frequency analyses of qualitative variables were done. 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.

Results

The study included 68 patients with chronic renal impairment. The mean age of the patients was 62.21±11.869 years. The male-to-female ratio was 31/37 (45.6% men and 54.4% women). The main comorbidity was hypertension - in 64 (94.12%) of the participants, and diabetes mellitus - in 40 (58.82%). Table 1 shows basic clinical and laboratory data of patients.

Parameter	Mean ± SD
Weight (kg)	79.80±15.405
SBP (mmHg)	141.76±17.593
DBP(mmHg)	88.97±8.833
Uric acid (μmol/L)	401.21±131.568
SCr (μmol/L)	208.40±140.327
Urea (mmol/L)	14.251±8.5618
eGFR (mL/min)	40.83±26.019
CysC (mg/L)	2.751 ± 1.139
sUmod (ng/ml)	122.9994±101.15855
RI	0.8357±0.12882

Table 1: Clinical characteristics and laboratory findings of the studied patients.
Note: SBP: systolic blood pressure; DBP: diastolic blood pressure; SCr: serum creatinine; eGFR: estimated glomerular filtration rate; CysC: cystatin C; sUmod: serum uromodulin; RI: resistive index; SD: standard deviation

The correlation between sUmod and different laboratory parameters is presented in Table 2.

Variables	r	p
SCr	- 0.720	< 0.0001
Urea	- 0.717	< 0.0001
Uric acid	- 0.296	0.017
CysC	- 0.353	0.004
eGFR	0.692	< 0.0001

Table 2: Analysis of correlations between various laboratory parameters and sUmod (r).
Note: SCr: serum creatinine; CysC: cystatin C; eGFR: estimated glomerular filtration rate

The correlation between sUmod and RI was r = - 0.353 (p=0.004).

In our study patients' sUmod levels were significantly correlated with SCr (r= -0.720, p<0.0001), urea (r= -0.717, p<0.0001), uric acid (r= -0.296, p=0.017) and CysC (r= -0.353, p=0.004). sUmod displayed inverse relationships with RI as well (r= -0.353, p=0.004). Correspondingly, a positive relationship with eGFR (r=0.692, p<0.0001) was found.

Discussion

Early diagnosis of CKD can reduse its progression to end-stage renal disease. Currently, SCr and urea level, CysC and eGFR are being used. Some of them seem to be insufficient and are not accurate enough for early diagnosis.

Therefore, new validated biomarkers are required for the assessment of CKD and its progression. We investigate the sUmod as a biomarker for CKD comparing it with all already established markers[9].

In our study sUmod ranges from 10 to 709.64 ng/mL with mean plasma levels 122.9994 ng/mL for all CKD stages combined. Scherberich et al. analyzed sUmod levels in 165 patients with various diseases potentially affecting the kidney and established values similar to ours - from 1 to 667 ng/mL with a median of 85 ng/mL[21].

Our results show that plasma Uromodulin expression significantly correlates with SCr levels. The relationship is strongly negative (r = -0.720). Fedak et al. found the same[22]. Their observational study included 170 patients with CKD stages 1 to 5, not treated by renal replacement therapy, and 30 healthy individuals used as a control group. sUmod concentrations were significantly lower among patients with CKD than in the controls, and displayed inverse relationship with creatinine (r= -0.39). Other authors established a similar finding - sUmod decreased with SCr's increase[23, 24].

sUmod concentrations are strongly negatively correlated with urea (r = -0.717). Risch et al. tested 289 participants and established a similar relationship (r= -0.30)[23]. Other previous studies also found a negative correlation between uromodulin and blood urea nitrogen[21, 24].

Reduced kidney excretion of uric acid that occurs in patients with CKD leads to elevated serum levels of uric acid and as a result - hyperuricemia[25,26]. We find negative correlation between uric acid and sUmod (r = -0.296).

sUmod concentrations are strongly negatively correlated with renal CysC (r = - 0.353). Our result is very close to that obtained from Risch et al. In their study sUmod also displayed inverse relationships with CysC (r= -0.42)[23]. Scherberich et al. and Steubl et al. found similar significant negative correlation (r= -0.862 and -0.79 respectively)[21,24].

In addition, our results show that sUmod correlates positively with eGFR (r = 0.692). Similar relationship was found in previous studies[10, 22-24, 27]. The result obtained by Lv et al. was very close to ours. They investigated 2652 CKD patients and found a positive correlation between sUmod and eGFR in multivariable linear correlation analysis (r=0.68, p<0.001)[12].

The relationship between elevated RI and renal function worsening has already been studied. The high RI (>0.70) was independent predictor of CKD progression[4, 28]. In our study we demonstrate a strong negative correlation between sUmod and RI (r = -0.353). Comparing sUmod with an instrumentally measured parameter, which is different from laboratory tests, once again confirms that uromoduline can be used as a marker of renal damage.

Study limitation

One limitation of our study is the relatively small sample size and single-center recruitment. Our cohort includes patients with different CKD stages but we did not compare the sUmod levels between them. Large cohort studies in the future are needed to confirm our findings.

Conclusions

Our study shows that sUmod levels significantly correlate with all alreadyestablished laboratory parameters used for evaluation of renal impairment. At the same time we determine strong negative correlation between sUmod and RI. Our findings support the view that sUmod can be used as a potential marker for CKD diagnosis and early assessment of disease progression.

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