

## Uromodulin Levels in Chronic Kidney Disease

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### Abstract

**Objectives:** Uromodulin is a protein exclusively produced by Kidney in tubular cells lining thick ascending limb (TAL) of the loop of Henle. It has been linked to water electrolyte balance, protective role against urinary tract infections and renal stone formation. To analyse its trend in Chronic Kidney Disease (CKD), its levels were analysed in serum and urine of patients at different stages of CKD.

**Methods:** CKD stage-1 and stage-2 (N=14), Stage-3 (N=24), Stage-4 (N=9), Stage-5 (N= 19) and controls (N=16) were recruited as per the National Kidney Foundation – Kidney Disease Outcome Quality Initiative guidelines. Uromodulin levels were analysed by enzyme linked immunosorbent assay (ELISA).

**Results:** Uromodulin levels both in serum and urine decreased with decreasing kidney function. Bivariate analysis within CKD group showed significant (p=0.001) positive correlation of urinary Uromodulin (spearman correlation [rs]=0.664) and serum Uromodulin (rs=0.855) levels with eGFR and significant (p=0.001) negative correlation of urinary Uromodulin (rs=-0.872) and serum Uromodulin (rs=-0.859) with serum creatinine levels. Serum Uromodulin levels in addition showed significant (p=0.001) negative correlation with phosphorus (rs = 0.523) and corrected calcium phosphorus product (rs=-0.484).

**Conclusion:** Serum and urine levels of Uromodulin decreased with increasing severity of CKD. Apart from its other protecting role, its reduction may accelerate pathophysiology associated with elevated calcium-phosphorus product in CKD.

**Keywords:** End-stage renal disease, Tamm–Horsfall protein, Thick ascending limb

### 1. Introduction

An interest in almost 60 years old protein; Uromodulin has been triggered by the identification of mutations in its gene; UMOD as cause of autosomal dominant kidney diseases, now referred to as Uromodulin-associated kidney diseases (UAKDs), presenting with tubulointerstitial fibrosis, defective urinary concentration, hyperuricaemia, gout, renal cyst and progressive renal failure.[1] In UAKDs, the key primary pathogenic event is a delayed intracellular trafficking of mutant UMOD, causing its intracellular accumulation.[2] In addition, the emerging epidemiologic evidence by genome wide association study that Uromodulin polymorphism may be associated with the development of end-stage renal disease (ESRD) regardless of the specific diagnosis also compels to study its trend in CKD. [3]

Uromodulin, also known as Tamm–Horsfall protein (THP), was first described by Tamm and Horsfall in 1950 as a urinary mucoprotein that is able to inhibit viral

agglutination [4]. Subsequently it has been also linked to water electrolyte balance, protective role against urinary tract infections and renal stone formation [5]. Uromodulin is produced in the thick ascending limb (TAL) of Henle's loop and early distal convoluted tubules of the nephron. [5] It is secreted in urine through proteolytic cleavage of the glycosylphosphatidylinositol (GPI) anchor. [5].

It is the most abundant protein excreted in the urine under physiological conditions. [4] Decreased urinary excretion of Uromodulin in parallel with the decrease in glomerular filtration rate (GFR) has been documented. [6-10] Although it is targeted to the apical membrane of TAL cells and secreted into the lumen, detectable levels are also found in venous blood. [11] In order to investigate the potential involvement of Uromodulin in CKD, paired samples of urine and serum were analysed for Uromodulin levels from CKD patients at different stages and correlated with estimated Glomerular Filtration Rate (eGFR) and with renal profile to determine its role in CKD.

## 2. Material and Methods

### 2.1 Study population

It comprised of 58 CKD patients. They were divided into 5 CKD stages as per National Kidney Foundation – Kidney Disease Outcome Quality Initiative (KDOQI) based on the estimated GFR (eGFR) and Urinary Microalbumin Creatinine Ratio (Albumin Creatinine Ratio [ACR]) greater than 30 mg/g Creatinine. [12] The eGFR was calculated using the equation Modification of Diet in Renal Disease (MDRD). CKD Stage-1 and stage-2 patients were with eGFR >90 mL/min/1.73 m<sup>2</sup> and 60-89 mL/min/1.73 m<sup>2</sup> respectively (N=14). Stage-3 were with eGFR 45-59 mL/min/1.73 m<sup>2</sup> (N=24), stage-4 were with eGFR 15–45 mL/min/1.73 m<sup>2</sup> (N=9) and stage-5 patients were with eGFR <15 mL/min/1.73 m<sup>2</sup> (N=19). Detailed information of patients regarding demographic status, clinical history, family history and medication taken was obtained on a Case Record Form. Controls (N=16) were healthy individuals with no other organic disease based on their clinical history and routine investigations. They were with normal renal profile, eGFR >90 mL/min/1.73 m<sup>2</sup> and ACR less than 30mg/g creatinine. All study participants were explained the study protocol and written informed consent was obtained. Study was approved by the Institutional Ethics Committee of Sir H. N. Hospital and Research Centre, Mumbai, (Letter dated 15<sup>th</sup> May 2013) which follows the ethical standards laid down by the ICMR's Ethical guidelines for biomedical research on human participants. Fasting peripheral blood samples were obtained from all the study participants.

### 2.2 Methods

Biochemical tests such as Blood Urea Nitrogen (BUN), Creatinine, Uric Acid, Total Protein, Albumin, Calcium (Ca) and Phosphorus (P) were performed using Konelab® i20 automatic analyzer. Globulin, Albumin to Globulin ratio, corrected Calcium (cCa) and cCaP products were calculated. Microalbumin, Total Protein, and Creatinine

were also estimated from Urine to calculate ACR and Protein Creatinine Ratio (PCR). Remaining serum and urine samples were aliquoted and stored at -20°C for estimation of Uromodulin from serum and urine by enzyme linked immunosorbent assay (ELISA) using commercially available kit (BioVendor Research and Diagnostic Products; CZECH Republic). The detection limit of the assay was 0.12ng/ml. The assay required dilution of 50X for serum and 2000x for urine samples of the controls. However, for patients of CKD stage 3-5 the dilution of the samples was not required to obtain the detection in the standard range (0.5-32ng/ml) of the assay kit.

### 2.3 Statistical Analysis

Results are expressed as frequency and percentages, mean ± standard deviation (SD) for parametric variables and median with inter quartile (25th/75th) ranges for non-parametric variables. For parametric variables, analysis of significance of difference between means of two groups was performed by student's unpaired t-test and non-parametric test, Mann Whitney U-test was applied for comparing significance between two medians. To quantify the extent of discrimination of Uromodulin levels between controls and CKD patients, reverse operating characteristic (ROC) analysis was carried out wherein the plot of sensitivity versus 1-specificity across varying cut-offs was generated. This gives a curve in the unit square called an ROC curve. Correlations were evaluated by Spearman's rank correlation test. A P value < 0.05 was considered statistically significant. Analyses were performed using statistical software SPSS (version 21.0, Chicago, IL).

## 3. Results

### 3.1 Demographic and Biochemical Tests

Controls were with normal eGFR and no Microalbuminuria. Patients of CKD stage 1, 2, 3, 4 and 5 matched in age and BMI (Table 1).

**Table 1: Demographic Data**

Demographic Parameter	Control Group (N = 16)	[CKD Stage 1&2] (N=14)	[CKD Stage 3] (N=15)	[CKD Stage 4] (N=9)	[CKD Stage 5] (N=19)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Age (years)	44.0± 5.54	57.6± 11.7	64.7 ± 7.74 <sup>NS</sup>	67.9± 11.5 <sup>NS</sup>	55.4± 7.54 <sup>NS</sup>
Male/ Female	9/7	8/6	11/4	2/7	12/7
BMI (kg/m <sup>2</sup> )	24.9 ± 4.99	24.9 ±4.40	25.8± 4.72 <sup>NS</sup>	26.3± 8.62 <sup>NS</sup>	23.9 ± 3.24 <sup>NS</sup>
BSA (m <sup>2</sup> )	1.64 ± 0.15	1.59 ± 0.11	1.76 ± 0.20 <sup>NS</sup>	1.54 ± 0.22 <sup>NS</sup>	1.6±.0.17 <sup>NS</sup>

NS Non-significant

CKD stage 3-5 compared against CKD stage 1 and 2

CKD stage-1 and stage-2 patients were compared against CKD stage-3, stage-4 and stage-5 patients. There was significant increase in creatinine in stage-3 (56.2%), stage-4 (145%/2.45 fold), stage-5 (432%/5.32 fold) and BUN in stage-3 (45.6%), stage-4(100%/2.0 fold), stage-5 (200%/3.0

fold) as compared to CKD stage-1 and stage-2 patients (Table 2). CKD stage-5 patients showed significant decrease in Total Protein (13.1%), Albumin (15.9%), Ca (11.8%) levels while significant increase in P (60.5%) and cCaP product (66.0%) as compared to CKD stage-1 and stage-2 patients (Table 2).

Table 2: Renal Profile

	Control Group (N = 16)	[CKD Stage 1&2] (N=14)	[CKD Stage 3] (N=15)	[CKD Stage 4] (N=9)	[CKD Stage 5] (N=19)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
BUN (mg/dl)	9.94 ± 3.31	11.5±2.74	16.7±5.4***	35.4±21.2***	45.6± 12.2***
Creatinine (mg/dl)	0.84± 0.19	1.05±0.14	1.64±0.30***	2.4± 0.45***	6.64± 1.56***
Total Protein (gm/dl)	7.73± 0.67	7.39 ± 0.66	7.22± 0.59 <sup>NS</sup>	6.78 ± 0.85 <sup>NS</sup>	6.42 ± 0.55***
Albumin (g/dl)	4.62 ± 0.47	4.13± 0.46	4.1± 0.44 <sup>NS</sup>	3.7± 0.58 <sup>NS</sup>	3.47 ± 0.36***
Globulin (gm/dl)	3.15 ± 0.43	3.25 ± 0.83	3.15 ± 0.64 <sup>NS</sup>	3.1± 0.53 <sup>NS</sup>	2.98 ± 0.53 <sup>NS</sup>
Albumin/Globulin	1.48 ± 0.22	1.34 ± 0.39	1.36 ± 0.37 <sup>NS</sup>	1.22± 0.29 <sup>NS</sup>	1.2 ± 0.28 <sup>NS</sup>
Estimated Glomerular Filtration Rate [eGFR] (ml min <sup>-1</sup> 1.73 m <sup>-2</sup> )	97.9 ± 18.2	72.4± 8.9	47.4±7.3***	23.8 ±4.4***	9.5 ± 2.5***
Uric Acid (mg/dl)	5.97 ± 1.05	5.51± 1.9	5.64± 1.93 <sup>NS</sup>	8.58± 2.26*	5.87 ± 2.2 <sup>NS</sup>
Calcium (mg/dl)	9.4 ± 0.31	10.1 ± 1.08	9.7 ± 0.68 <sup>NS</sup>	9.4 ± 0.81 <sup>NS</sup>	8.9± 0.81*
cCa (mg/dl)	9.06±0.49	9.96±1.14	9.22± 1.73 <sup>NS</sup>	9.62 ± 0.56 <sup>NS</sup>	9.36±0.91 <sup>NS</sup>
Phosphorous (P) (mg/dl)	3.7± 0.77	3.84± 0.67	3.44 ± 0.85 <sup>NS</sup>	4.36 ± 0.95 <sup>NS</sup>	6.16± 3.06**
Corrected Ca x P product (mg <sup>2</sup> /dl <sup>2</sup> )	33.2± 6.91	35.6± 13.9	31.9±10.3 <sup>NS</sup>	41.8 ± 8.91 <sup>NS</sup>	59.1± 32.8**
	Median (25th/75th)	Median (25th/75th)	Median (25th/75th)	Median (25th/75th)	Median (25th/75th)
Urinary Microalbumin to Creatinine Ratio -ACR (mg/g Creatinine)	4.25 (1.17/7.84)	45.5 (25.4/97.1)	69.4*** (18.9/209)	782*** (233/1274)	2032*** (1328/3363)
Urinary Total Protein to Creatinine Ratio -PCR (mg/g Creatinine)	47.12 (33.2/59.2)	178 (103/336)	259*** (114/808)	1678*** (874/2047)	3974*** (3685/8400)

NS Non-significant, \*p<0.05, \*\*P<0.01, \*\*\*p<0.0001  
CKD stage 3-5 compared against CKD stage 1 and 2

3.2 Uromodulin Levels

In control the serum and urinary Uromodulin levels were 123 (79.6/93.8) ng/ml and 58 (27.4/190) mg/gm creatinine respectively. Serum Uromodulin levels decreased significantly (p<0.001) by 35.2%, 73.9% and 88.8% (Figure 1a) and Urinary Uromodulin decreased significantly (p<0.001) by 55.7%, 94.6% and 96.52% (Figure 1b) in CKD stage-3, stage-4 and stage-5 respectively as compared to CKD stage-1 and stage-2 groups.

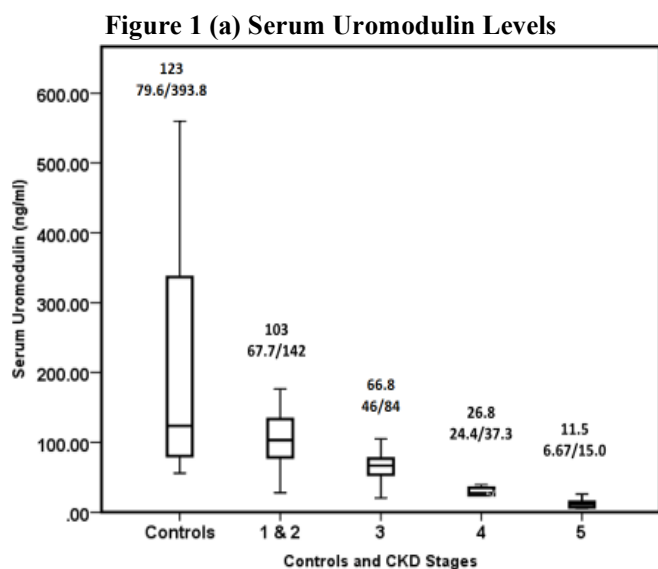
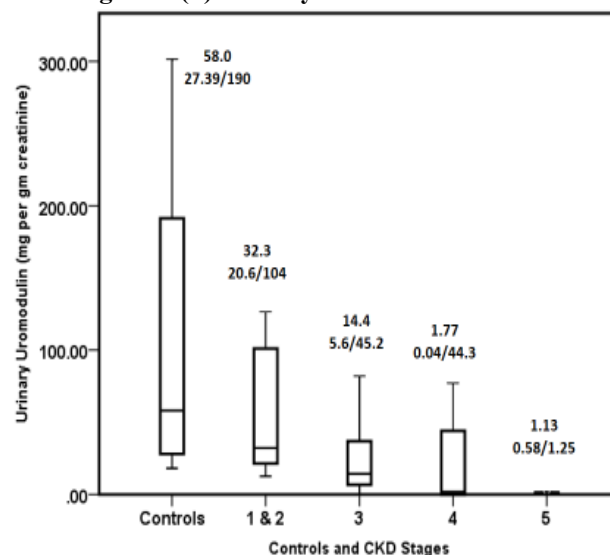
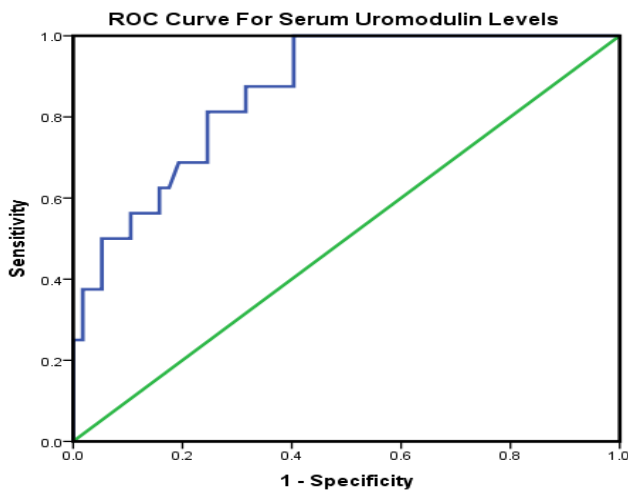


Figure 1 (b) Urinary Uromodulin Levels

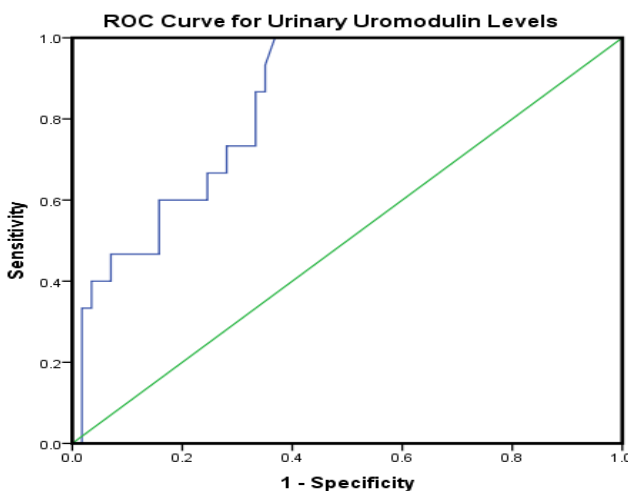


According to Receiver Operating Characteristics (ROC), the area under curve (AUC) represents the probability that a randomly selected patient will have a lower or higher test result than a randomly selected control. The ROC curve of serum Uromodulin demonstrated AUC of 0.862 (SE-0.045) (95% CI-0.774-0.951, p=0.001) (Figure 2a) and that of urinary Uromodulin showed AUC of 0.839 (SE-0.49) (95% CI 0.774-0.934, p=0.001) (Figure 2b). Thus AUC of both serum and urinary Uromodulin suggested that on average, a patient will have a lower Uromodulin levels than approximate 80% of the controls.

**Figure 2a: ROC for Serum Uromodulin**



**Figure 2b: ROC for Urinary Uromodulin**



**3.3 Correlation Data**

CKD patients combined of all stages, demonstrated a positive correlation between serum and urinary Uromodulin levels ( $r=0.696$ ,  $p=0.001$ ). Positive correlation of serum and urinary Uromodulin levels was observed with eGFR, Albumin, Total Protein and negative correlation with serum Creatinine, ACR and PCR. Serum Uromodulin levels in addition showed significant negative correlation with P and cCaP product (Table 3).

**Table 3: Bivariate Correlation within CKD group**

	Spearman Correlation	
	Serum Uromodulin	Urine Uromodulin
<b>eGFR</b>	0.855**	0.664**
<b>Albumin</b>	0.548**	0.364**
<b>Total Protein</b>	0.564**	0.483**
<b>Serum Creatinine</b>	-0.859**	-0.872**
<b>ACR</b>	-0.632**	-0.381**
<b>PCR</b>	-0.720*	-0.605**
<b>Phosphorus</b>	-0.523**	0.254 <sup>NS</sup>
<b>Corrected Calcium Phosphorus product</b>	-0.484**	-0.225 <sup>NS</sup>
<b>Uric Acid</b>	0.216 <sup>NS</sup>	-0.280*

NS Non-significant, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.0001$

**4. Discussion**

In the present study, levels of Uromodulin exclusively produced by kidney were found to decrease both in urine and serum with decreased kidney function, correlating positively with eGFR and negatively with serum creatinine levels.

Uromodulin has been shown to interact with and activate specific components of the immune system, protect against UTI and thus may act as a signalling molecule for renal tubular damage. Damage of the TAL and cells distal to the TAL results in Uromodulin leakage into the interstitium, which causes a pro-inflammatory response. When this condition is mild, the response is appropriate, and tubular damage is repaired. Sustained leakage of Uromodulin into the interstitium would most likely cause an immune reaction against Uromodulin-secreting cells themselves, resulting in a decrease of TAL cell numbers, eventually to the point where neither urinary nor serum Uromodulin are detected [13]. Serum Uromodulin level measurement in venous blood however, do not necessarily mirror the amount of Uromodulin in the renal interstitium, especially due to the high affinity binding of this protein with many immune cells and immune components. [13] However, measuring both serum and urinary Uromodulin, provides a way where one can delineate patients specifically with Uromodulin TAL component in their pathology i.e. if the TAL cells themselves are damaged or when their numbers are decreased, both leading to altered secretion of Uromodulin. [14]

Our results corroborated with several others wherein decreased urinary Uromodulin levels have been reported in CKD patients. [6-10] Pracjczar *et al* [14] have reported that eGFR correlated positively with urinary Uromodulin levels but negatively with serum Uromodulin levels. However, they have further reported that in a subgroup of patients with lowest eGFR, urinary as well as serum Uromodulin levels were lowest. This suggests that even serum Uromodulin levels correlated positively with low eGFR where Uromodulin secreting cells were almost destroyed. Recently, Risch *et al* [15] have also documented significant differences ( $p<0.001$ ) in serum Uromodulin among the four groups according to different kidney function stages. Further in their study, serum Uromodulin displayed inverse relationships with creatinine ( $r=-0.39$ ) and a positive relationship with eGFRcysC ( $r=0.38$ ,  $p<0.001$ ) similar to that found in the present study.

Among the correlation findings of the present study, serum Uromodulin levels showed negative correlation with P and cCaP product. The total serum calcium x phosphate product is an indicator of the risk of mineral crystallisation in soft tissues and is one of the contributors for mineral bone disease. Although often supersaturated with mineral salts such as calcium phosphate and calcium oxalate, normal urine possesses an innate ability to inhibit formation of harmful crystals. This inhibitory activity is due to Uromodulin. [16].

Supporting this, UMOD knock-out mice models have shown a propensity for increased calcium crystal formation in the urinary tract and a reduced ability to inhibit the adhesion of calcium oxalate monohydrate crystals to renal epithelial cells. [16] In another study, in a mice lacking Uromodulin, it was observed that in addition to increased concentration of urine phosphorus and calcium phosphate super saturation, there was interstitial deposits of calcium phosphate within the renal papillae. [17] Thus, in addition to decreased renal function due to TAL damage, patients with severely attenuated Uromodulin may become more susceptible to pathophysiology associated with elevated calcium-phosphorus product.

Thus present study explored the trend of Uromodulin; a protein only of kidney at different stages of CKD. The limitation of the study is small sample size. However, serum and urinary levels of this protein reduced significantly with increasing severity of the disease. CKD group combined of all stage showed significant positive association of urinary as well as serum Uromodulin levels with eGFR and negative association with serum creatinine levels. Further, loss of protection due to reduced levels Uromodulin may be one of the factors in worsening pathophysiology associated with increase calcium phosphorus imbalance in CKD.

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