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Original Research Article

# ROLE OF SERUM UROMODULIN AS A BIOMARKER OF DECLINE IN RENAL FUNCTION IN CHRONIC KIDNEY DISEASE

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ABSTRACT

**Background:** Uromodulin (Tamm–Horsfall protein), the most common protein in normal urine present in a bundant quantity, is increasingly considered as a potential biomarker relevant to kidney function and tubular reserve, chronic kidney disease and hypertension. A reduced number of tubular cells, is paralleled by reduced urinary and serum concentrations of uromodulin. Therefore, uromodulin might represent a promising biomarker for CKD. **Aim:** To evaluate the use of serum uromodulin as a biomarker in chronic kidney disease patients.

**Material and Methods:** In an observational study, on 100 subjects with 50 normal healthy and 50 with CKD 1 – 5 stages patients attending Nephrology department in a tertiary care hospital for two years were enrolled. Lipid profile, Serum calcium, proteins, creatinine and serum Uromodulin along with e GFR was investigated. Data was analyzed using SPSS 16 and by using t test, ANOVA and correlation coefficients.

**Results:** Mean age of the study participants was 47.47 ± 10.66 years. And 71% were male. On comparison between cases and controls, Serum Uromodulin, calcium, HDL was lower and other lab findings were significantly more in Cases than in controls. Serum Uromodulin was significantly decreased with severity of CKD.

**Conclusion:** A comparative analysis of serum uromodulin with e GFR shows that eGFR is positively correlated with uromodulin. Lower serum uromodulin reflects a decline in kidney function. Thus serum uromodulin is a helpful marker in diagnosis of CKD at a nearly stage. The estimation of serum uromodulin level may aid in early diagnosis of kidney dysfunction and also in predicting the progression of disease.

**Keywords:** Biomarker, Chronic Kidney disease, Renal function, Serum Uromodulin.

## INTRODUCTION

In 2015 the Global Burden of Disease (GBD) study estimated 1.2 million deaths due to chronic kidney disease, and estimated an increase of 32% since 2005. It was estimated that 2.3–7.1 million people died with end-stage kidney disease without access to dialysis in 2010.[1,2] International Society of Nephrology's Kidney Disease Data Center reported that prevalence of Chronic Kidney disease (CKD) as 17% . States like Andhra Pradesh, Odisha have high levels of CKD of unknown etiology (CKDu), which is a chronic interstitial nephropathy with insidious onset and gradual progression. In Southern India, Chennai and Vishakhapatnam districts have higher prevalence.[3,4]

Uromodulin is a glycosylphosphatidylinositol-anchored protein synthesized in tubular cells of the ascending limb of Henle's loop and released into the urine by proteolytic cleavage.[5–7] In the urinary system, the renal defensin uromodulin exerts anti-lithogen, anti-infective and immunomodulatory functions.[8–10] Mutations of the uromodulin-coding

gene may cause severe kidney damage, such as tubulocystic kidney disease, recurring urinary tract infections, familial juvenile hyperuraemic nephropathy and congenital nephrolithiasis.[11,12] Even small changes in uromodulin concentration or function, e.g. caused by uromodulin loci variants, may trigger or accelerate kidney disease.[13–16] Serum uromodulin (sUmod) is a promising kidney tissue biomarker that does not directly depend on glomerular filtration but mirrors tubular function and nephron mass.[17]

Serum creatinine(SCr) is an important tool in assessing renal function by estimated GFR. But creatinine concentration may be within the normal range inspite of the GFR being reduced. Creatinine concentration is thus a relatively insensitive index of mild renal functional impairment. Many previous studies suggested that impairment in tubular function was associated with decreased kidney function, so reduction in serum uromodulin level could be used as an early biomarker. Uromodulin can differentiate healthy persons from persons with various stages of CKD. One way to reduce the burden of chronic kidney disease would be early intervention.[7] Therefore, it is important to explore biomarkers that can diagnose the disease earlier.

The prevalence of CKD is increasing over the past few decades and simultaneously cardiovascular risk is also increasing due to diabetes & hypertension, whereas biomarkers for predicting CKD is not well established. Henceforth the present study is planned to measure the serum uromodulin level as a predictor of renal function in CKD.

**Aim and Objectives**

To evaluate the use of serum uromodulin as a biomarker in chronic kidney disease patients.

To correlate serum uromodulin with serum creatinine and eGFR.

## MATERIAL AND METHODS

A Single centre based case control observational study was conducted on 100 subjects i.e., 50 healthy volunteers taken as controls and 50 patients of stages 1 -5 of CKD ascases. Cases were the patients attending Nephrology outpatient clinic at department of Nephrology, Kurnool Medical college & General govt. Hospitals Kurnool. The study was approved by the institutional ethical committee. Detailed information of patients regarding demographic status, clinical history, family history and medication taken was obtained. Controls (N=50) were healthy individuals with no other organic disease based on their clinical history and routine investigations with normal renal profile and eGFR >90 ml/min/1.73m2. Patients with chronic kidney disease stages 1-5, aged 18 -60 years were included and Pregnant and lactating women, patients on Immunosuppressive drugs, Cancer and after renal transplantation/dialysis are excluded from the study.

CKD stages have been categorized as per NKF-KDOQI as follows

- Stage 1 - eGFR > 90 ml/min/1.73 m2
- Stage 2 - eGFR 60-89 ml/min/1.73 m2
- Stage 3 - eGFR 45-59 ml/min/1.73 m2
- Stage 4 - eGFR 15–45 ml/min/1.73 m2
- Stage 5 - eGFR < 15 ml/min/1.73 m2

Sample collection: For the study, 5ml of venous blood sample was collected from all the study participants and serum was separated after centrifugation at 3000 rpm for 15 minutes 23 and aliquoted into an eppendorf tube and stored at -20°C and not thawed until the batch was analyzed for uromodulin. Routine blood investigations like sugar, urea, creatinine, lipid profile, calcium, total protein, albumin were performed on the same day of blood collection in semi auto analyser.

Methods: The measurement of serum uromodulin was performed by ELISA using Elabscience commercial reagent kits like ROBONIK Elisa Reader and Washer Human THP (Tamm– Horsfall Glycoprotein) ELISA Kit by Sandwich ELISA principle, with a biotin labelled antibody, a calibration range of 0 to 100ng/ml, and a detection limit of 1.56- 100 ng/ml. Estimation of the analyte was done as follows

Blood Sugar - GOD POD method
Blood Urea - GLDH -urease method
Serum Creatinine - Jaffes Method
Serum Total Cholesterol - CHOD-PAP method
Serum Triglycerides - Glycerol 3 Phosphate oxidase method
Serum HDL - Phosphotungstic Acid method
Serum Calcium - Arsenazo method
Serum Total protein - Biuret method
Serum Albumin - Bromocresol green method.

Statistical analysis: Data entry was done using Microsoft Excel 2013 and analysis using SPSSV16. Qualitative data was represented in frequencies and percentages and quantitative data was represented in mean and standard deviation. T test was used to find the significance between two quantitative variables. ANOVA was used to find the significance between more than three groups. Pearson correlation coefficient was used to find the significant correlation between two quantitative variables. ROC analysis was done to find the diagnostic accuracy of Uromodulin in detecting CKD.

## RESULTS

In the present observational study, the mean age of the study population was 47.47 ± 10.66 years. Among cases, the mean age was 49.06 ±12.76 years. Among control, mean age was 45.88 ±7.86 years. In the present study, 71% were male and 29% were female. Among cases majority were male i.e. 74% and among controls 68% were male. [Figure 1]

Table 1 shows the comparison between lab findings among cases and controls. The mean uromodulin among cases was 89.95 ± 57.99 and among control

it was 241.72 ± 30.88. Among cases, mean uromodulin, mean HDL, mean calcium, mean total protein, mean albumin was significantly lower than in controls and this observation was statistically significant. Among cases, mean RBS, mean Urea, mean creatinine, mean triglyceride was significantly higher than in controls and this observation was statistically significant. There was no significant difference observed with relation to mean total cholesterol between the study groups as the pvalue calculated to be>0.05.

In the present study, a significantly lower Uromodulin levels were observed in the later stages of CKD compared to earlier stage of CKD. The mean uromodulin and CKD stage I patients was178.50±4.10, among CKD stage II patients was 177.19 ± 23.40, among CKD stage III was 114.06 ± 19.27 and among CKD stage IV was 41.93 ± 12.64 and among CKD stage Vwas31.13 ± 8.00. This observation was statistically significant. (Table 2)

Among cases, a significant positive correlation was observed with Pearson correlation coefficient t(r)=0.94, with p value 0.0001*between uromodulin and eGFR levels. (Figure 2) Among cases, a significant negative correlation was observed with Pearson correlation coefficient (r)=-0.74, with p value 0.0001*between uromodulin and creatinine levels. (Figure 3) Figure 4 depicts the ROC analysis showing the significance of Uromodulin as biomarker. On ROC analysis, uromodulin at a cut off value of<185 has a sensitivity of 96% and specificity of 96% to predict CKD.

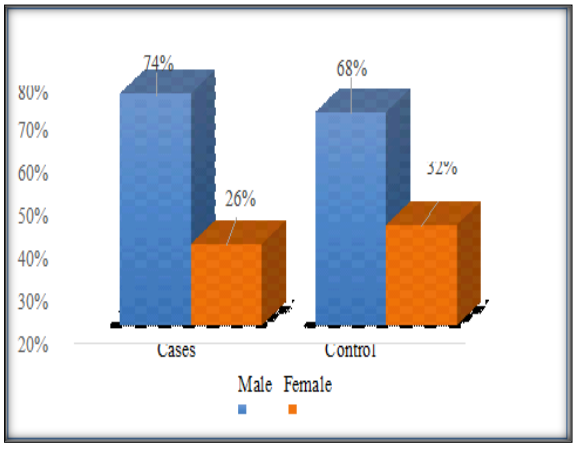


**Figure 1: Gender wise distribution**

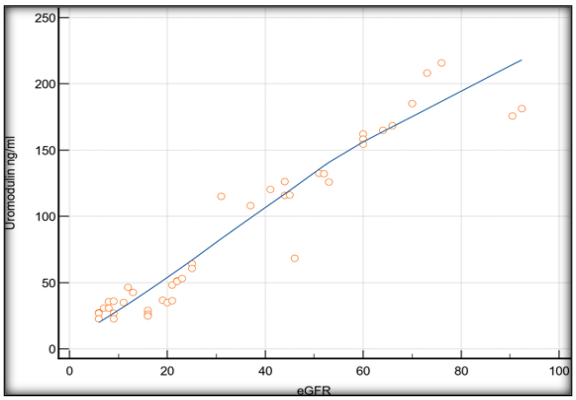


**Figure 2: Correlation between Uromodulin and GFR in Cases**

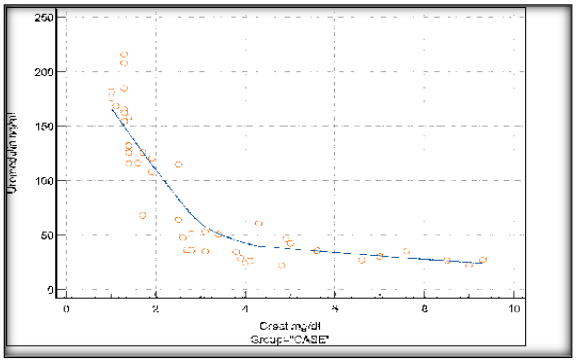

**Figure 3: Correlation between Uromodulin and Creatinine in Cases**

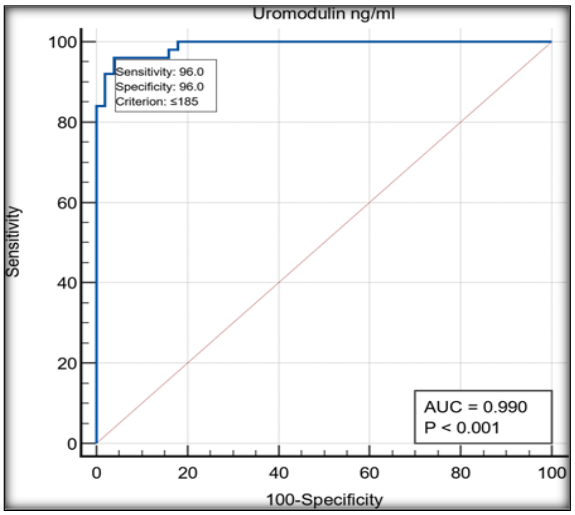


**Figure 4: ROC curve for uromodulin**

**Table 1: Lab findings among Cases and Controls**

| | Case | Control | p |
|---|---|---|---|
| Mean Uromodulin | 89.95±57.99 | 241.72±30.88 | 0.0001* |
| Mean RBS | 158.42±55.80 | 94.90±10.98 | 0.0001* |
| Mean Urea | 87.82±62.47 | 24.83±9.74 | 0.0001* |
| Mean Creatinine | 3.17 ±2.27 | 0.74±0.11 | 0.0001* |
| Mean Total cholesterol | 169.62±48.22 | 183.08±23.74 | 0.07 |
| Mean Triglycerides | 174.08±54.21 | 121.90±44.50 | 0.0001* |
| Mean HDL | 36.22±4.18 | 44.57±44.50 | 0.0001* |
| Mean Calcium | 8.19±0.86 | 9.53±0.53 | 0.0001* |
| Mean Totalprotein | 6.76 ±0.49 | 7.59 ±0.22 | 0.0001* |
| Mean Albumin | 3.56 ±0.47 | 4.48 ±0.24 | 0.0001* |

**Table 2: Lab parameter sin the CKD group**

| | CKD Stage | | | | | P value |
|---|---|---|---|---|---|---|
| | I(n=2) | II(n=8) | III(n=16) | IV(n=14) | V (n=10) | |
| Age | 40.50±6.36 | 37.25±15.48 | 53.50±10.12 | 49.14±11.48 | 53.00±11.96 | 0.02* |
| RBS | 146.00±14.14 | 140.13±79.74 | 141.25± 62.83 | 167.36± 44.77 | 190.50± 19.74 | 0.18 |
| Urea | 42.50±3.54 | 48.38±12.86 | 62.65±28.46 | 89.07±63.58 | 167±63.21 | 0.0001* |
| Creatinine | 1.00±0.00 | 1.29±0.08 | 1.75±0.35 | 3.75±1.40 | 6.59±1.93 | 0.0001* |
| Cholesterol | 205.00± 7.07 | 161.63± 43.79 | 154.13± 64.83 | 175.86± 36.89 | 185.00± 33.29 | 0.39 |
| Triglyceride | 128.00± 11.31 | 150.00± 31.98 | 167.81± 62.03 | 186.93± 65.37 | 194.60± 28.71 | 0.24 |
| HDL | 41.00±1.41 | 39.50 ±2.56 | 35.81 ±2.48 | 35.43 ±5.36 | 34.40 ±4.33 | 0.03* |
| Calcium | 9.85±0.07 | 9.26±0.48 | 8.60±0.24 | 7.60±0.42 | 7.21±0.17 | 0.0001* |
| Totalprotein | 7.35±0.07 | 7.27±0.33 | 7.00±0.27 | 6.59±0.24 | 6.09±0.25 | 0.0001* |
| Albumin | 4.15±0.07 | 4.13±0.17 | 3.87±0.15 | 3.24±0.19 | 2.97±0.13 | 0.0001* |
| Uromodulin | 178.50±4.10 | 177.19±23.4 | 114.1±19.3 | 41.93±12.64 | 31.13 ±8.00 | 0.0001* |
| eGFR | 91.45±1.34 | 66.13 ±6.29 | 43.50 ±7.00 | 18.79 ±5.28 | 8.70±2.58 | 0.0001* |

## DISCUSSION

Chronic Kidney Disease has become a significant public health problem and the increasing number of CKD patients in recent years is due to the higher incidence of non-communicable diseases, especially diabetes and hypertension. It remains asymptomatic till a test age and intervention become in effective to reduce the progression of the disease.[18] Therefore, it is essential to explore new biomarkers that can help in early diagnosis as well a stop redict the prognosis of CKD. In this study, we have assessed serum uromodulin level in healthy people and patients with different stages of CKD.

In our study, plasma uromodulin confirmed the previously documented,[19] inverse behavior compared to other biomarkers resulting in lower plasma uromodulin concentrations at advanced stages of CKD. From the current pathophysiological understanding this circumstance is due to the fact that uromodulin may not be, like creatinine and BUN, an indirect marker for glomerular filtration but a direct marker for the amount of intact tubular cells of the ascending limb (where it is exclusively produced) and therefore may represent a marker for the number of remaining functional nephrons/renal tissue/tubular secretion. This is a novel approach to measure “kidney function,” potentially helping the treating physician to assess the remaining renal mass and therefore kidney function in the phase when conventional markers /glomerular filtration fails to indicate deterioration of kidney function.

In the present study among cases, a significant negative correlation was observed with Pearson correlation coefficient (r)=-0.74, with p value 0.0001* between uromodulin and creatinine levels. This finding was similar to the study findings of Fedak et al[20], Genov et al[21], Rischetal[19] In Risch et al[19] study sUmod also displayed inverse relationships with CysC (r= - 0.42)(23). Scher berich et al.[22] and Steubl et al.[23]found similar significant negative correlation (r=-0.862and-0.79 respectively)

In the present study, among cases, a significant positive correlation was observed with Pearson correlation coefficient (r)= 0.94, with p value 0.0001* between uromodulin and eGFR levels. Genov et al,[21] results show that sUmod correlates positively with eGFR (r =0.692). Similar relationship was found in previous studies. [10, 24, 25] 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)[26]

In 2016, Steubl et al,[23] published the results of a study in which they have demonstrated that plasma uromodulin levels were significantly lower in patients with CKD compared with those without kidney disease and gradually decreased with the progression of renal disease. They reported a strong negative correlation between plasma uromodulin levels and markers of renal retention (serum creatinine, cystatin C, and blood urea nitrogen) and a strong positive correlation between plasma uromodulin levels and eGFR. Similar correlation was found in our study also. Serum uromodulin concentration gradually decreased with impairment of kidney function.

In our study, with increase severity of CKD or stages of CKD, the serum Uromodulin levels gradually decreased. Thus significantly predicts serum uromodulin as biomarker and predictor of prognosis and our findings were similar to other studies.[20,21] In another study, Leiherer et al. stated that lower levels of serum uromodulin were independently associated with the decline of kidney function[26]

In our study, the lipid abnormalities were Hyper triglyceridemia and low HDL in CKD patients. V pandya. A. et al also demonstrated that Hyper triglyceridemia and low HDL was the abnormality found in CKD patients who studied lipid profile in CKD patients.[27] In the study ROC curve analysis was done to differentiate between healthy persons and patients with CKD. In ROC, area under the curve (AUC) is 0.99 (95% CI0. 946 to 1.000, P= <

0.0001) at an optimal cut off of 185 ng/ml with 96 % sensitivity and 96% specificity. Hence uromodulin can be used as a early marker for CKD. The main limitation of our study was the Small sample size, Large cohort studies are needed to confirm the clinical values of uromodulin in CKD and Other chronic kidney disease markers like cystatin would have been measured. The role of uromodulin can be validated and explored as an effective, easy and early biomarker of CKD for timely diagnosis and treatment.

## CONCLUSION

The present study observed that uromodulin may play a significant role in CKD progression. A serum uromodulin value of less than 178 ng/ml even when serum creatinine is normal may indicate an early stage of CKD and thus help in early detection CKD. This can help alert for early preventive measure. So the estimation of serum uromodulin level may aid in early diagnosis of kidney dysfunction and also in predicting the progression of disease.

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