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Role Of Uromodulin As A Predictor Of Decline In Renal Function.

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ABSTRACT

Uromodulin is a 95 KD protein, released from the basolateral region of tubular cells from where it reaches the interstitial space of the kidneys and detectable levels are usually present in blood. Since it is secreted & synthesised by tubular epithelial cells, its level is decreased in kidney dysfunction. This study aims to find out the role of uromodulin as a biomarker and its correlation with serum creatinine and eGFR in chronic kidney disease patients. A total of 50 controls & 50 cases of CKD patients from stage 1-5 were chosen. Serum uromodulin was measured by ELISA. Sugar, urea, creatinine and lipid profile were measured using standard kits. Graph pad prism 8.2.1 used for statistical analysis. Pearson correlation & unpaired t tests were used. Serum uromodulin level was significantly reduced in patients with CKD ($p<0.0001$). Uromodulin displayed negative correlation with creatinine and positive correlation with eGFR ($p <0.01$).In this study, we have found that serum uromodulin can be used as an early marker of renal dysfunction and also that it predicts the progression of disease.

Keywords: CKD, eGFR, Renal function, Uromodulin.

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INTRODUCTION

Chronic kidney disease (CKD) is one of the major public health burdens globally and is also one of the leading causes of fatal cardiovascular diseases. Chronic kidney disease may remain asymptomatic till late stage and can progress to End Stage Renal Disease (ESRD) over time.

Chronic kidney disease (CKD) is defined as kidney damage or GFR < 60 ml/min/1.73 m^2 for at least three months irrespective of the cause. Kidney damage is defined by pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. Diabetes and Hypertension are the most common causes of the CKD in India[1].

Based on estimated GFR (eGFR), CKD has been categorized into five stages (CKD 1 -5).If GFR >90 ml/min/1.73 m^2 - stage 1, GFR 60-89 ml/min/1.73 m^2- stage 2, GFR 45–59 ml/min/1.73 m^2-Stage 3 A , GFR 30–44 ml/min/1.73 m^2 -Stage 3 B, GFR 16-29 ml/min/1.73 m^2- stage 4,GFR < 15 ml/min/1.73 m^2 - Stage 5. Serum creatinine (sCr) serves as an important tool in assessing renal function by estimated GFR. But creatinine concentration may be within the reference range in spite of the GFR being reduced. Creatinine concentration is thus a relatively insensitive index of mild renal functional impairment.

This may lead to delay in diagnosing the disease and so delay in treatment. Therefore, it is necessary to detect the disease at the earliest [2-4].

Various biomarkers are increasingly considered as useful tools for the diagnosis, classification, and prognosis of kidney diseases. Uromodulin levels in serum might be a useful biomarker for renal tubular function.

Uromodulin is a 95 KD protein. It is synthesised by the thick ascending limb and the distal tubule cells of kidney. It is an abundant protein excreted in urine of healthy individuals. Uromodulin is released from the basolateral region of tubular cells , from where it reaches the interstitial space of the kidneys and detectable levels are usually present in blood[5].

Uromodulin gene (UMOD) was confirmed as one of the most important loci associated with end-stage renal failure. Serum uromodulin concentration is found to be gradually decreased with decline in kidney function and in chronic kidney disease patients [6].

Many previous studies suggested that impairment in tubular function was associated with reduced kidney function and so decrease in serum uromodulin level could be used as an early biomarker. Uromodulin can differentiate between healthy persons and patients with all 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.

Hence the present study is on measurement of serum uromodulin levels in healthy persons and patients with all stages of chronic kidney disease (CKD).

Aims

- To evaluate the use of serum uromodulin as a biomarker in chronic kidney disease patients.
- To assess the role of uromodulin as a predictor of renal function in chronic kidney disease.

Objectives

- Estimation of eGFR in chronic kidney disease patients of the study population.
- To correlate serum uromodulin with serum creatinine.
- To correlate serum uromodulin with eGFR.

MATERIALS AND METHODS

A case control study was conducted during December 2017 – May 2018 at Government Mohan Kumaramangalam Medical College Hospital, Salem. Informed consent was obtained from all patients. All procedures concerning patients were permitted by the Institutional Ethical Committee. The study

consisted of 50 healthy people taken as controls. 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/1.73m^2.50 patients of stages 1 -5 of CKD treated in the Nephrology outpatient clinic at department of Nephrology, Government Mohan Kumaramangalam Medical college & Hospitals were taken as cases.

The cases were divided into 5 CKD stages as per National Kidney Foundation – Kidney Disease Outcome Quality Initiative (KDOQI) based on the estimated GFR (eGFR).The eGFR was calculated using CKD EPI formula.

CKD

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

Inclusion criteria were Patient with Age 18 -60 years & chronic kidney disease stages 1-5.

Exclusion criteria were Pregnancy and lactating women, Those with Immunosuppressive drugs, Systemic autoimmune disease, Cancer, After renal transplantation/dialysis.

All study participants were explained about the study protocol.

Sample Collection

For the study, 5ml of venous blood sample was collected from all the study participants and the following investigations were performed. Sugar, urea, creatinine, lipid profile, calcium, total protein and albumin were performed on the same day of blood collection in auto analyser. The measurement of serum uromodulin was performed by ELISA using Elabscience commercial reagent kits.

Methods

Sugar was estimated by GOD POD method, urea was estimated by GLDH -urease method, creatinine by Jaffes Method, total cholesterol by CHOD-PAP method, triglycerides by Glycerol 3 Phosphate oxidase method, HDL by Phosphotungstic Acid method, calcium by Arsenazo method, total protein by Biuret method, albumin by Bromocresol green method. Uromodulin ELISA assay was 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 .

Statistics

Statistical analysis was done by using Graph Pad Prism 8.2.1. and the p value less than 0.05 was considered statistically significant . Continuous data has been expressed as Mean and Standard Deviation. Unpaired t test was used to compare the mean value of biochemical parameters between controls and cases. Pearson Correlation was used for finding the correlation of uromodulin with biochemical parameters like creatinine, eGFR, total Protein, albumin and calcium.

The receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic accuracy of serum uromodulin levels to differentiate between healthy persons and CKD patients for determining CKD stages.

RESULTS

Uromodulin, Sugar, Urea, Creatinine, Total Protein, Albumin, Lipid profile, Calcium levels in serum were estimated in controls and cases. eGFR values were calculated using CKD EPI formula. Sex distribution among cases and controls were shown in Table 1, Fig1.

Table 2,Fig2, shows the comparison between serum uromodulin levels in controls and cases, uromodulin level was significantly reduced in patients with CKD in comparison with controls (P<0.0001).

As per Table 3, Fig3 , on comparing serum creatinine level between controls and cases, there was statistically significant elevation of creatinine level in cases compared to controls (p=0.0001 <0.05).

Lipid profile data is shown in Table & Fig 4-6. When the Total Cholesterol level between controls and cases were compared , the difference was not statistically significant (p= 0.548 >0.05) and there was a statistically significant elevation of Triglyceride level in cases when compared to controls (p=0.0001 <0.05) and statistically significant decrease in HDL levels in cases compared to controls (p= 0.0001 <0.05).

As per the Table &Fig 7 -9, Total Protein , Albumin and Calcium levels were compared between controls and cases. Showed statistically significant decrease in Total Protein, Albumin and Calcium levels in cases compared to controls (p= 0.0001 <0.05).

Bivariate correlation analysis between serum uromodulin and eGFR is Shown in Figure 11 and found positive correlation between serum uromodulin and eGFR (r = 0.962 , P<0.01) , which indicates the concentration of serum uromodulin was closely related with eGFR.

Also correlation of serum uromodulin with total protein, albumin, calcium was determined by Pearson Correlation Analysis. All stages of CKD The correlation between creatinine and serum uromodulin was shown in Figure 10, the scatter plot suggests an inverse relationship, indicating decrease in serum uromodulin level with impaired kidney function.

Patients showed a positive correlation between uromodulin and total protein, albumin,calcium (p < 0.01).ROC curve for serum uromodulin shown in Fig 13. It shows 98 % sensitivity,99 % specificity(P< 0.0001).

Figure 1: Sex Distribution In Controls And Cases

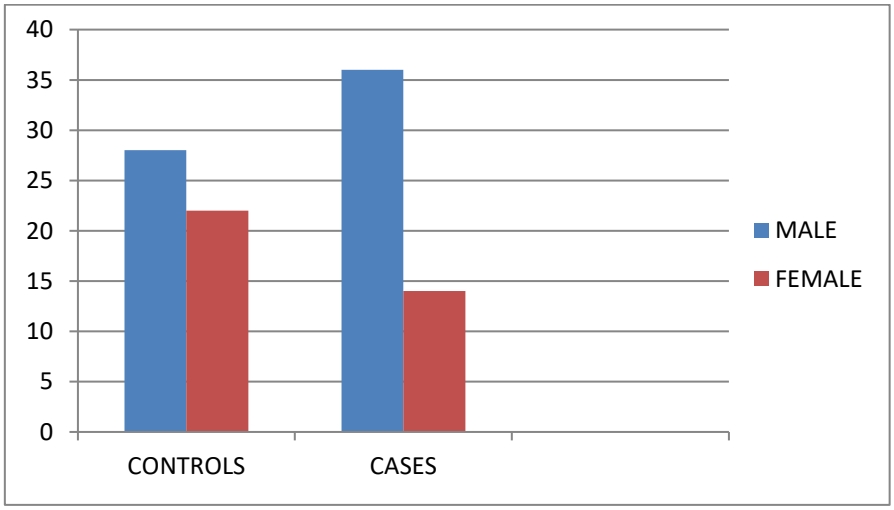

Figure 2: Serum Uromodulin In Controls And Cases

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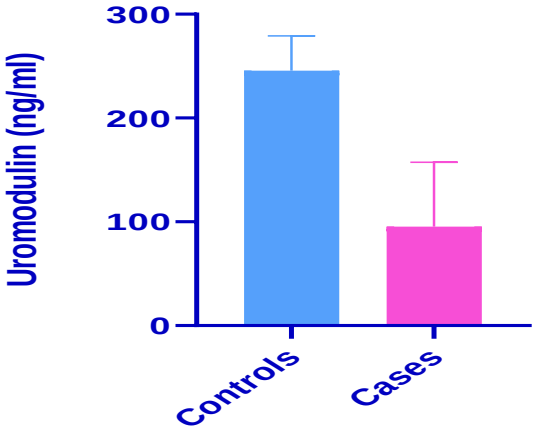

Figure 3: Creatinine In Controls And Cases

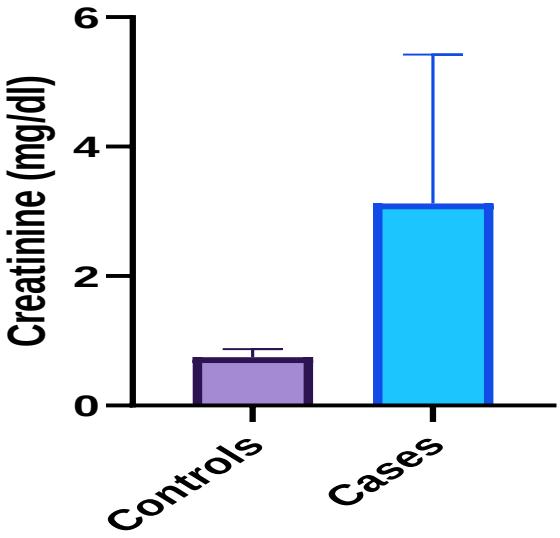

Figure 4: Total Cholesterol In Controls And Cases

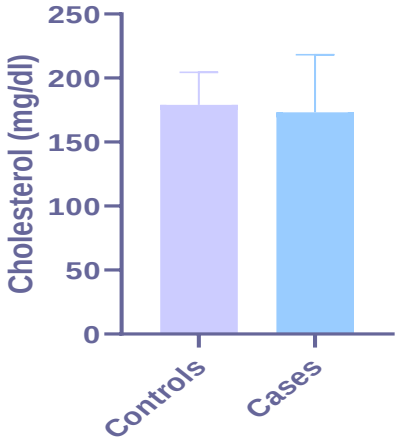

Figure 5: Triglyceride In Controls And Cases

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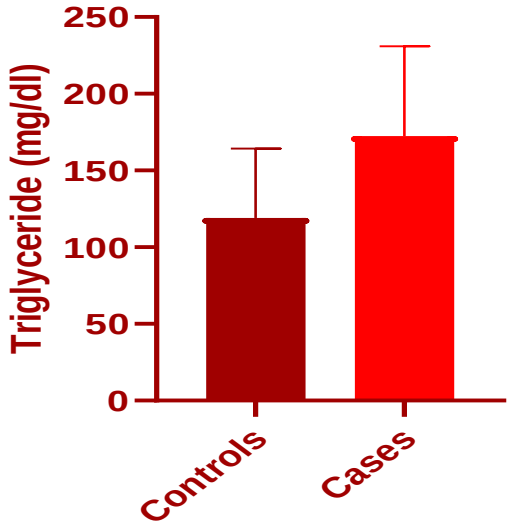

Figure 6: HDL In Controls And Cases

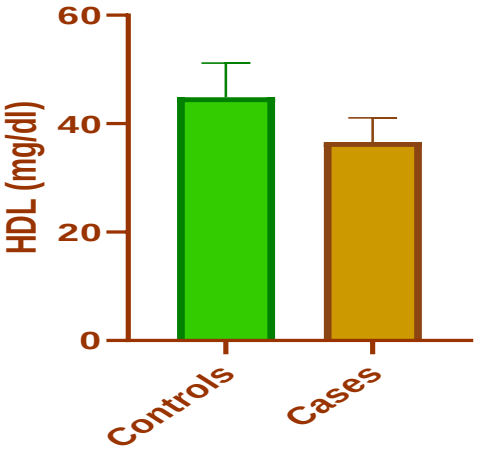

Figure 7: Total Protein In Controls And Cases

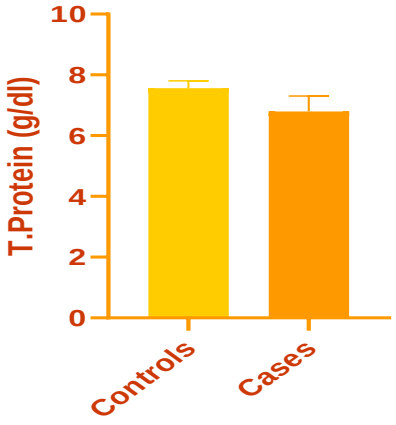

Figure 8: Serum Albumin In Controls And Cases

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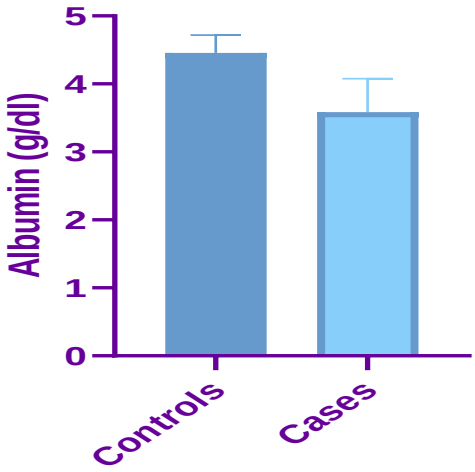

Figure 9: Calcium In Controls And Cases

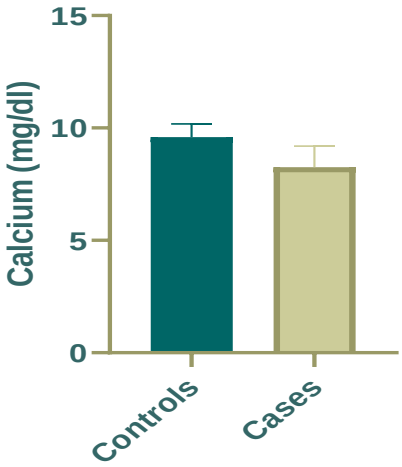

Figure 10: Correlation of Creatinine with Uromodulin

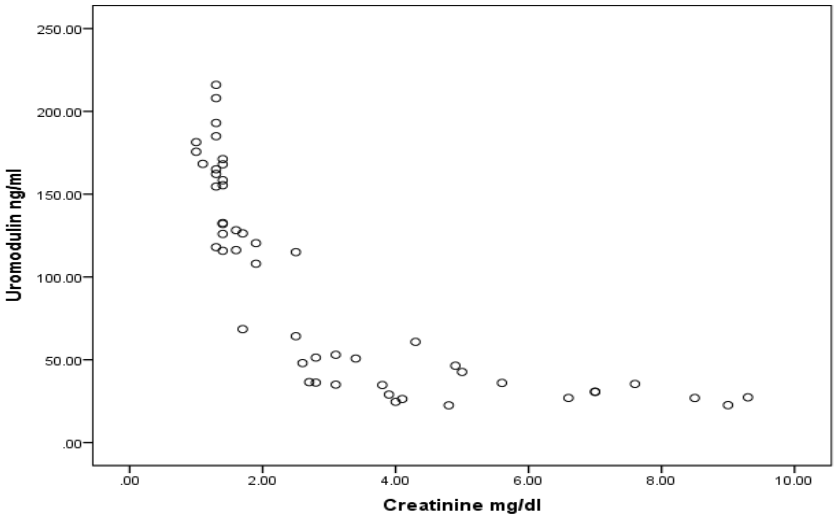

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Figure 11: Correlation of eGFR with Uromodulin

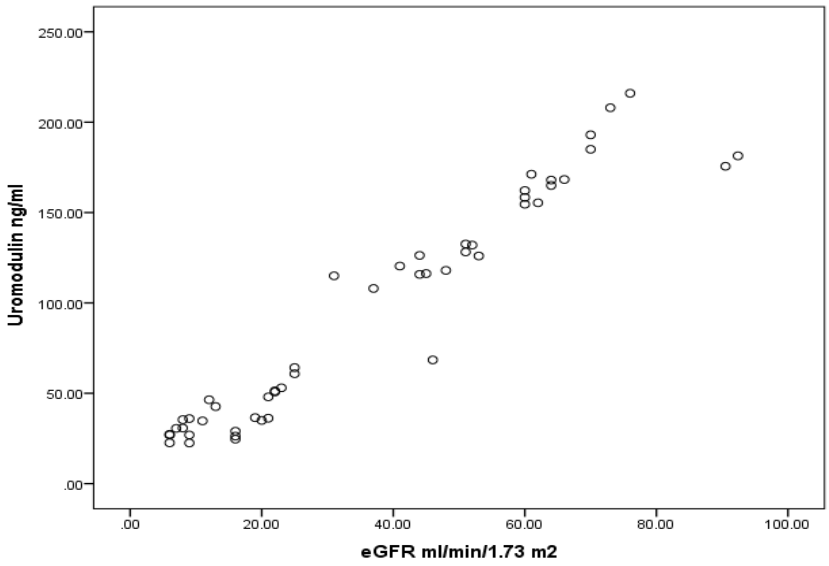

Figure 12: Box whisker plot showing uromodulin levels in controls & cases

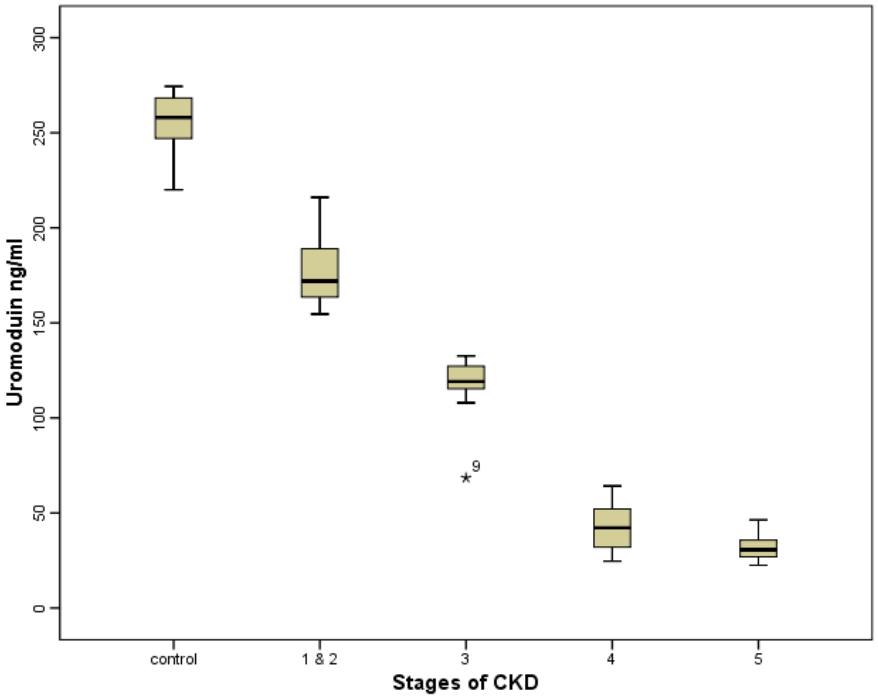

Figure 13: ROC curve for Uromodulin

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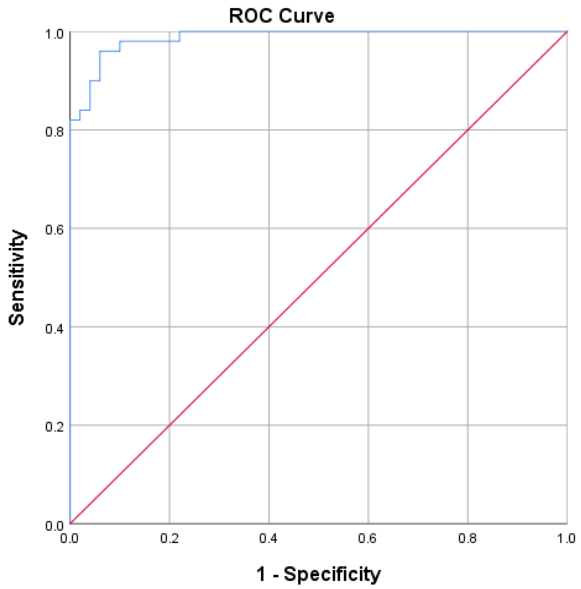

Table 1: Sex Distribution In Controls And Cases

	MALE	FEMALE
CONTROLS	28	22
CASES	36	14

Table 2: Comparison Of Serum Uromodulin Level In Controls And Cases

UROMODULIN ng/ml	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	245.6	33.52	p = 0.0001
CASES (n=50)	95.35	61.98	(< 0.05 significant)

Table 3: Comparison Of Creatinine Level In Controls And Cases

CREATININE mg/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	0.74	0.12	p=0.0001
CASES (n=50)	3.12	2.30	(<0.05 significant)

Table 4 : Comparison Of Total Cholesterol Between Controls And Cases

T.CHOLESTEROL mg/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	179.16	20.12	p= 0.548
CASES (n= 50)	173.3	45.05	(not significant)

Table 5: Statistical Analysis Of Triglycerides In Controls And Cases

TRIGLYCERIDE (mg/dl)	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n= 50)	118 .9	45.2	p = 0.0001
CASES (n= 50)	172.42	58.59	(<0.05significant)

Table 6: Comparison Of HDL In Controls And Cases

HDL mg/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	44.80	6.3	p=0.0001
CASES (n=50)	36.60	4.4	(<0.05 significant)

Table 7: Comparison Of Total Protein In Controls And Cases

TOTAL PROTEIN g/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	7.56	0.23	p = 0.0001
CASES (n=50)	6.79	0.50	(<0.05 significant)

Table 8 : Comparison Of Serum Albumin In Controls And Cases

SERUM ALBUMIN g/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	4.45	0.26	p=0.0001
CASES (n=50)	3.58	0.49	(<0.05 significant)

Table 9: Comparison Of Calcium In Controls And Cases

CALCIUM mg/dl	MEAN	SD	STATISTICAL INFERENCE
CONTROLS (n=50)	9.59	0.58	p=0.0001
CASES (n=50)	8.2	0.92	(<0.05 significant)

Table 10: Pearson Correlation Between Uromodulin And Creatinine In Study Group

		Uromodulin	Creatinine
Uromodulin	Pearson Correlation	1	-.764**
	Sig. (2-tailed)		.000
	N	50	50
Creatinine	Pearson Correlation	-.764**	1
	Sig. (2-tailed)	.000	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 11: Pearson Correlation Between Uromodulin And eGFR

		Uromodulin	eGFR
Uromodulin	Pearson Correlation	1	.962**
	Sig. (2-tailed)		.000
	N	50	50
eGFR	Pearson Correlation	.962**	1
	Sig. (2-tailed)	.000	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 12: Pearson Correlation Between Uromodulin And Protein

		Uromodulin	T.Protein
Uromodulin	Pearson Correlation	1	.815**
	Sig. (2-tailed)		.000
	N	50	50
T.Protein	Pearson Correlation	.815**	1
	Sig. (2-tailed)	.000	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 13 : Pearson Correlation Between Uromodulin And Albumin

		Uromodulin	Albumin
Uromodulin	Pearson Correlation	1	.907**
	Sig. (2-tailed)		.000
	N	50	50
Albumin	Pearson Correlation	.907**	1
	Sig. (2-tailed)	.000	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 14 : Pearson Correlation Between Uromodulin And Calcium

		Uromodulin	Calcium
Uromodulin	Pearson Correlation	1	.882**
	Sig. (2-tailed)		.000
	N	50	50
Calcium	Pearson Correlation	.882**	1
	Sig. (2-tailed)	.000	
	N	50	50
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 15: Bivariate Correlation Within CKD Group Pearson Correlation

	Serum Uromodulin
eGFR	0.962**
Creatinine	- 0.764**
Total Protein	0.815**
Albumin	0.907**
Calcium	0.882**

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 late stage and intervention become ineffective to reduce the progression of the disease[10] .Therefore, it is essential to explore new biomarkers that can help in early diagnosis as well as to predict the prognosis of CKD. In this study ,we have assessed serum uromodulin level in healthy people and patients with different stages of CKD.

Uromodulin is a glycoprotein exclusively synthesized in renal tubular epithelial cells. Most of this protein is released into urine, but significant basolateral release of uromodulin is secreted to the tubulo interstitium which is detected in the blood. Uromodulin is being studied as a marker for kidney function and is found to differentiate between patients without CKD and with CKD of all stages by simply assessing serum concentrations, at a reasonable level of sensitivity and specificity.

In our study, serum uromodulin level is decreased in patients with CKD. This result is supported by Dominic stebl et.al , in which they observed that serum uromodulin concentrations were significantly higher in individuals without CKD compared to patients with CKD of all stages[11].During abnormal kidney function/kidney damage, the decline in uromodulin concentration is caused by the reduction in function and/or reserve of the tubules since it is secreted by epithelial cells of tubules.

It has been found that tubulointerstitial injury is closely linked to progression of renal disease. Low serum uromodulin parallels a structural derangement of the TAL and may drop due to altered intracellular processing and reduced basolateral exocytosis ,which is proportional to the reduced quantity of intact TAL cells. Kidney damage is characterized by epithelial atrophy and tubular loss of both cortical and medullary segments and also parallel changes of the vascular microarchitecture. Interstitial vascular remodelling may influence the structural integrity of tubular cells, and change glomerular morphology and haemodynamics, which would link serum uromodulin levels with eGFR (CKD stages) [9].

In our study, the decrease in serum uromodulin level with decreasing kidney function , which is correlated positively with eGFR and negatively with serum creatinine .

Our results are consistent with many studies. Risch et al [12] have also documented that serum uromodulin displayed lower concentrations with decreasing kidney function. Further in their study, serum Uromodulin showed inverse relationships with creatinine, a positive relationship with eGFR similar to that found in the present study.

Pracjczer et al. have also reported that eGFR correlated positively with serum uromodulin which is similar to that found in the present study. Chronic thick ascending limb (TAL) damage led to a reduction in the number of TAL cells and serum uromodulin concentrations, indicating uromodulin playing an active role in the development of CKD [6]. In another study, Leiherer et al. stated that lower levels of serum uromodulin were independently associated with the decline of kidney function[8].

In 2016, Steubl et al [7] 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, which correlates with our results.

Serum uromodulin concentration gradually decreased with impairment of kidney function .We performed ROC curve analysis ((fig 13) to differentiate between healthy persons and patients with CKD. In ROC, area under the curve (AUC) is 0.9872(95 % CI 0.9724-1.000 , p= < 0.0001) at an optimal cut off of 175.8 ng/ml with 98 % sensitivity and 99% specificity. Hence uromodulin can be used as an early marker for CKD.

In our study, the lipid abnormalities are Hypertriglyceridemia and low HDL in CKD patients .Similar studies by VPandya.A.et al demonstrated that Hypertriglyceridemia and low HDL was the abnormality found in CKD patients who studied lipid profile in CKD patients, which correlates with our present study[13].

Hypertriglyceridemia in kidney disease due to both abnormal production and reduced catabolism of triglycerides, which is mediated by down regulation of lipoprotein lipase (LPL) and hepatic triglyceride lipase, which have the primary physiologic function of cleaving triglycerides into free fatty acids for energy production or storage and also an increase in the plasma apo C-III/apoC-II ratio, apo C- III is an inhibitor of LPL. Chylomicron remnants and IDL cholesterol accumulate because of the decrease in the catabolism of triglycerides.HDL cholesterol is considered as “good” cholesterol, because of reverse cholesterol transport processes. In patients with CKD, HDL cholesterol maturation is impeded due to LCAT deficiency (the enzyme important for the esterification of free cholesterol in HDL), so that Apo A-1 level decreases. Since ApoA-I is the primary protein constituents of HDL, its deficiency can, in part, contribute to the overall reduction of plasma HDL in CKD. Deficiency of Apo A-I in chronic renal failure is due to their diminished gene expression in the liver. ApoA-1 deficiency can impair the binding of HDL to ATP binding cassette transporter A-1 and this impaired step causes a dysfunction in free cholesterol efflux from macrophages to HDL cholesterol hence accumulation of free cholesterol in macrophages produces foamy cells in vessels and causes formation of atherosclerotic plaques [14].

Already established renal markers have limitations in detecting the kidney function in earlier CKD stages, particularly when half of the filtration capacity is lost. Our results indicate that higher serum uromodulin concentration is associated with better kidney function. A comparative analysis of serum uromodulin with eGFR shows, eGFR is positively correlated with uromodulin. Thus indicates lower serum uromodulin reflects a decline in kidney function and leads to CKD. All the above studies agrees that serum uromodulin is a helpful marker in diagnosis of CKD at an early stage .

CONCLUSION

Uromodulin is synthesised by the cells of the thick ascending limb of the loop of henle, hence lower uromodulin serum levels may reflect a reduction in the number or function of these cells in CKD. Serum uromodulin showed a positive association with kidney function and behaved in a manner opposite that of conventional retention markers of kidney function.

It uniquely allows the identification of early stages of CKD when conventional biomarkers of kidney function are still within normal range. From this present study, we have found that serum uromodulin can be used as a earlier marker of kidney function and also predict the progression of disease.

Limitations

The limitation of the study is small sample size and Other chronic kidney disease markers like cystatin was not measured. Large cohort studies are needed to confirm the clinical values of uromodulin in CKD.

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