RESEARCH PAPER

"Association of Serum Uromodulin and α-Klotho biomarkers with severity of chronic kidney disease"

Khwayish Sharma[1], Ashish Sharma[2], Pooja Patidar[3*], Shivali sharma[4], Deepti Bhatt[5]

[1]Khwayish Sharma: PG Student, Department of Biochemistry, Geetanjali Medical College & Hospital, Geetanjali University, Udaipur, Rajasthan, India,313002. Email: sharmakhwayish@gmail.com
[2]Ashish Sharma: MBBS, MD, Professor, Department of Biochemistry, Geetanjali Medical College & Hospital, Geetanjali University, Udaipur, Rajasthan, India, 313002. Email: dr.ashishsharma@geetanjaliuniversity.com
[3*]Pooja Patidar: Senior Demonstrator, Department of Biochemistry, Geetanjali Medical College & Hospital, Geetanjali University, Udaipur, Rajasthan, India, 313002.Email: poojapatidar85@gmail.com, ORCID id: 0009-0001-5686-9739
[4]Shivali Sharma: Senior Demonstrator, Department of Biochemistry, Geetanjali Medical College & Hospital, Geetanjali University, Udaipur, Rajasthan, India, 313002.
Email: shivalisharma0077@gmail.com
[5]Deepti Bhatt : Senior Demonstrator, Department of Biochemistry, Geetanjali Medical College & Hospital, Geetanjali University, Udaipur, Rajasthan, India, 313002.
Emai: drdeeptibhatt2191@gmail.com

Corresponding Author: Pooja Patidar,
Senior Demonstrator, Department of Biochemistry, Geetanjali Medical College, Geetanjali University, Udaipur, Rajasthan, India, 313002, ORCID id: 0009-0001-5686-9739
Email: poojapatidar85@gmail.com

ABSTRACT

Background: Chronic kidney disease is frequently diagnosed late because traditional biomarkers have limited sensitivity for early renal damage. Serum uromodulin and α-klotho have emerged as potential indicators of early tubular injury and kidney function.

Materials and Methods: A cross-sectional study was conducted on patients diagnosed with CKD stages 1–5 according to KDIGO 2012 guidelines. Serum uromodulin and α-klotho levels were measured by ELISA method. Routine biochemical parameters were measured by automated analyzers and eGFR was calculated by the CKD-EPI equation.

Statistical Analysis: Data were analyzed using SPSS v 26.0. Normality was tested using the Kolmogorov–Smirnov test. One Way ANOVA and Kruskal-Wallis test was applied. Correlation analysis assessed relationships between variables. A p-value < 0.05 was considered statistically significant.

Results: Serum uromodulin and α-klotho levels showed a significant progressive decline with advancing CKD stages ($p < 0.001$). Both biomarkers demonstrated a strong positive correlation with eGFR ($r > 0.7$, $p < 0.001$) and negative correlations with serum creatinine and urea. Iron and ferritin levels were also altered across CKD stages. ROC curve analysis showed a higher diagnostic performance for serum uromodulin compared with α-klotho, with AUC values of 0.921 and 0.854, respectively. The sensitivity was 87.5% for uromodulin and 77.5% for α-klotho at cut-off values ≤144.6 ng/ml and ≤4.1 ng/ml, respectively.

Conclusion: Serum uromodulin and α-klotho serve as promising early biomarkers for detecting CKD and monitoring disease progression. Their combined assessment may enhance diagnostic accuracy and facilitate timely therapeutic interventions to prevent end-stage renal disease. Associated with CKD severity and renal function in this cross-sectional cohort.

Keywords: Chronic kidney disease, Uromodulin, α-Klotho, eGFR.

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INTRODUCTION: A gradual decline in renal function is known as chronic kidney disease (CKD). Infections, autoimmune illnesses, diabetes, hypertension, cancer, and toxic substances can all cause the kidneys to lose their normal function, especially excretory and regulatory functions. CKD is rapidly acquiring epidemic proportions worldwide and is on the threshold of becoming a serious health issue[1].CKD affects more than 850 million people globally and is predicted to rank fifth overall in terms of years of life lost by 2040[2]. More than three months of renal damage or a GFR of less than 60 ml/min/1.73 m2 are markers of chronic kidney disease (CKD)[3]. For it to improve the effectiveness of therapy, biochemical markers are necessary for appropriate diagnosis, evaluation of risk, and treatment application. The mass of the protein uromodulin (Umod) is 95 KD. The thick ascending limb and distal tubule cells of the kidney produce it. Urine from healthy individuals often contains this protein. Uromodulin is released by the basolateral region of tubular cells, goes to the interstitial space of the kidney, and is usually present in blood at detectable

*Author for Correspondence: poojapatidar85@gmail.com

concentrations[4]. The uromodulin gene (UMOD) has been identified as one of the major genes associated with end-stage renal failure. Serum uromodulin levels have been demonstrated to gradually decline in patients with chronic renal disorders and those with deteriorating kidney function[5].Alpha-Klotho (α-Klotho), a transmembrane (TM) protein, occurs mainly in the kidney's distal tubular cells[6]. Based on the pathogenic mechanism of α-Klotho deficiency and declining blood levels of α-Klotho even in stage 1 of CKD, Xianjin B et al. observed that α-Klotho is a sensitive indicator for impaired renal function and an alternative predictor of the risk of overall mortality of CVD events related to CKD. On the other hand however, the loss of α-Klotho associated with kidney disease has a significant effect on the progressive development of CKD and CVD[7]. α-Klotho is regarded as a highly sensitive indicator for early detection of chronic kidney disease (CKD), demonstrating a decline in serum levels even in the early stages of the disease, indicating high sensitivity. However, its specificity may vary depending on the research population and cut-off value utilized, indicating that additional clinical testing may be required to confirm the diagnosis because it may not always clearly differentiate CKD from other illnesses[8].Creatinine tests quantify the level of creatinine phosphate in the blood and identify impaired renal function. An increase in the serum is an indication of renal disease, while urea and creatinine are reliable markers of a healthy kidney. The most extensively used indicators to evaluate renal function are BUN and serum creatinine. Due to the invasive nature of blood collection for serum analysis, patients experience nervousness and discomfort as a result of blood loss from repetitive blood sampling, which may raise the risk of infections caused by blood for both patients and medical professionals. Therefore, an affordable diagnostic test that offers an accurate assessment of the disease status and stages and is beneficial to patients as well as physicians will be required. The most often used indicators to evaluate renal and chronic kidney disease (CKD) status are urea and creatinine [9-11].

"End-stage renal disease (ESRD) is regarded as a most serious outcome"[12]. "Anaemia is an undesirable and frequent consequence of chronic kidney disease (CKD) and causes increased morbidity and mortality"[13]. "The prevalence of anaemia in stage 3–5 CKD was 12.0%, and it has recently been noted that anaemia begin to develop early in the progression of CKD"[14]. In addition to chronic blood loss, decreased intestinal absorption, inflammation, infection, decreased red blood cell (RBC) survival, hyperparathyroidism, bone marrow depression, folate, iron, and vitamin B12 deficiencies, the main cause of anaemia in stages 4-5 of CKD is the reduced production of erythropoietin by failing kidneys[15,16]. Patients with chronic renal disease frequently suffer from anaemia as the outcome of iron insufficiency. Poor food intake, occult bleeding, or an imbalance between the actual iron supply and the iron requirements of the erythroid marrow are some of the major causes of iron insufficiency. Anaemia in chronic kidney disease (CKD) can also be caused by uremic inhibitors such as parathyroid hormone and inflammatory cytokines[17].

Although previous studies have reported associations between serum uromodulin, α-Klotho, and kidney function, data from Indian CKD populations remain limited, particularly across different CKD stages. The present study aimed to evaluate the relationship of serum uromodulin and α-Klotho with CKD severity and eGFR in a cross-sectional cohort of patients with CKD stages 1–5. We additionally compared the diagnostic discrimination of these biomarkers across CKD stages and explored their association with conventional biochemical markers of kidney function and iron metabolism.

MATERIALS AND METHODS:

This single-centre, observational cross-sectional study was conducted in the Department of Nephrology after obtaining ethical approval from the Human Research Ethics Committee (Ref: GU/HREC/EC/2024/2438). The study included 90 adult patients with an established diagnosis of chronic kidney disease (CKD) across all stages. Comprehensive clinical histories were recorded for all participants, and written informed consent was obtained before to their inclusion in the study.

Included those who were older than 18 years of age, and who did not have a history of atherosclerotic cardiovascular disease. Exclusion criteria included a history of heart failure, chronic inflammation, Patients on renal replacement therapy, Treatment with Immunosuppressive drugs, Systemic autoimmune disease and after renal transplantation.

Diagnostic Criteria and Staging of Chronic Kidney Disease:

The diagnosis and staging of chronic kidney disease were carried out in accordance with the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines. For each participant, the estimated glomerular filtration rate (eGFR) was calculated using a standard, validated equation. Patients were then classified into six CKD stages based on their corresponding eGFR values[18].

- **Stage 1:** GFR ≥90 mL/min/1.73 m2 with evidence of kidney damage
- **Stage 2:** GFR 60 to 89 mL/min/1.73 m2
- **Stage 3a:** GFR 45 to 59 mL/min/1.73 m2
- **Stage 3b:** GFR 30 to 44 mL/min/1.73 m2
- **Stage 4:** GFR 15 to 29 mL/min/1.73 m2
- **Stage 5:** GFR <15 mL/min/1.73 m2 or treatment by dialysis.

Sample Collection and Analysis: Venous blood samples were obtained from all participants using standard phlebotomy techniques. The collected samples were processed in the central laboratory for the measurement of a comprehensive panel of biochemical parameters. Serum uromodulin and serum α-klotho were measured by a commercially available ELISA kit.

According to the manufacturer's instructions. Other serum biomarkers were determined by a fully automated Cobas Pro Analyzer.

STATISTICAL ANALYSIS: All data were analyzed using SPSS statistical software (version 20.0). Quantitative variables were expressed as mean ± standard deviation (SD). Differences in biomarker levels across the various stages of chronic kidney disease were assessed using one-way analysis of variance. For variables that did not follow a normal distribution, the Kruskal–Wallis test was applied. Associations between serum biomarker levels and estimated glomerular filtration rate (eGFR) were examined using Pearson's or Spearman's correlation coefficients, as appropriate. A two-tailed p-value of < 0.05 was considered statistically significant for all analyses.

RESULTS:

Demographics and Clinical Characteristics of the Study Cohort:

A total of 90 patients with chronic kidney disease (CKD) were included in the study. The mean age of the participants was 50.90 ± 14.33 years. Males constituted 61.1% (n=55) of the study population, whereas females accounted for 38.9% (n=35).

As summarized in Table 1**,** the distribution of participants across CKD stages was uneven and predominantly represented advanced disease. Stage 5 CKD comprised the largest subgroup (42.2%, n=38), followed by Stage 4 (22.2%, n=20) and Stage 3b (14.4%, n=13). Early-stage CKD was relatively underrepresented, with only 4.4% (n=4) in Stage 1, 11.1% (n=10) in Stage 2, and 5.6% (n=5) in Stage 3a**.**The distribution of participants was uneven across CKD stages, with a predominance of Stage 4–5 disease (64.4%). Consequently, estimates for early-stage CKD should be interpreted cautiously due to the limited number of participants in Stages 1–3a.The predominance of advanced CKD stages should be considered when interpreting the findings, as estimates for early-stage disease are based on a limited number of participants**.**

Table 1: Demographic and Clinical Characteristics of Study Population.

Variables	Age (Years) (n= 90)	Male (n= 55)	Female (n= 35)	Stage 1 (n= 4)	Stage 2 (n=10)	Stage 3a (n= 5)	Stage 3b (n= 13)	Stage 4 (n= 20)	Stage 5 (n= 38)
	50.90±14.33	61.1%	38.9%	4.4%	11.1%	5.6%	14.4%	22.2%	42.2%

As shown in Table 2, serum α-Klotho and uromodulin concentrations progressively decreased across increasing CKD stages.Mean serum α-Klotho levels declined from 7.03 ± 1.12 ng/mL in Stage 1 to 1.66 ± 0.93 ng/mL in Stage 5 ($p < 0.001$). Similarly, mean serum uromodulin levels decreased from 255.77 ± 39.87 ng/mL in Stage 1 to 36.16 ± 22.69 ng/mL in Stage 5 ($p < 0.001$).

In contrast, conventional renal function markers showed progressive increases with advancing CKD severity. Mean serum urea increased from 24.62 ± 0.47 mg/dL in Stage 1 to 189.74 ± 50.28 mg/dL in Stage 5, while serum creatinine increased from 0.95 ± 0.97 mg/dL to 7.91 ± 3.70 mg/dL (both $p < 0.001$). Both urea and creatinine demonstrated strong inverse relationships with renal function. Serum urea showed a strong negative correlation with eGFR ($r = -0.914$, $p < 0.001$), and serum creatinine was similarly negatively correlated with eGFR ($r = -0.783$, $p < 0.001$).

Table 2: Mean Levels of Serum Biomarkers across CKD Stages.

Variables	Stage 1 (n=4)	Stage 2 (n=10)	Stage 3a (n=5)	Stage 3b (n=13)	Stage 4 (n=20)	Stage 5 (n=38)	p value
α-Klotho (ng/ml)	7.03±1.117	5.13±1.35	4.41±0.85	4.05±1.21	3.34±1.23	1.66±0.93	<0.001*
Uromodulin (ng/ml)	255.77±39.87	184.27±44.96	155.24±25.78	119.11±28.66	73.23±13.47	36.16±22.69	<0.001*
Urea (mg/dl)	24.62±0.47	32.71±3.2	43.4±3.577	68.02±12.85	109.15±16.45	189.74±50.28	<0.001*
Creatinine (mg/dl)	0.95±0.97	1.23±0.316	1.48±0.27	2.42±0.76	4.17±1.65	7.91±3.704	<0.001*

*** Statistically significance**

These findings demonstrate a significant association between lower serum uromodulin and α-Klotho concentrations and greater CKD severity. However, because of the cross-sectional study design, the observed associations should not be interpreted as evidence of disease progression or predictive utility.

As shown in figure 1, Receiver operating characteristic (ROC) analysis was performed to assess the ability of serum uromodulin and α-Klotho to discriminate advanced CKD (Stages 4–5) from earlier CKD stages (Stages 1–3b). Serum uromodulin demonstrated excellent discriminatory performance with an AUC of 0.921 (95% CI: 0.864–0.978, $p < 0.001$). At a cut-off value of ≤144.6 ng/mL, the sensitivity and specificity were 87.5% and 90.0%, respectively. Serum α-Klotho demonstrated good discriminatory performance with an AUC of 0.854 (95% CI: 0.734–0.973, $p < 0.001$). At a cut-off value of ≤4.1 ng/mL, sensitivity and specificity were 77.5% and 90.0%, respectively. These findings indicate that both biomarkers are associated with CKD severity, with serum uromodulin showing greater discriminatory ability than serum α-Klotho in this study population.

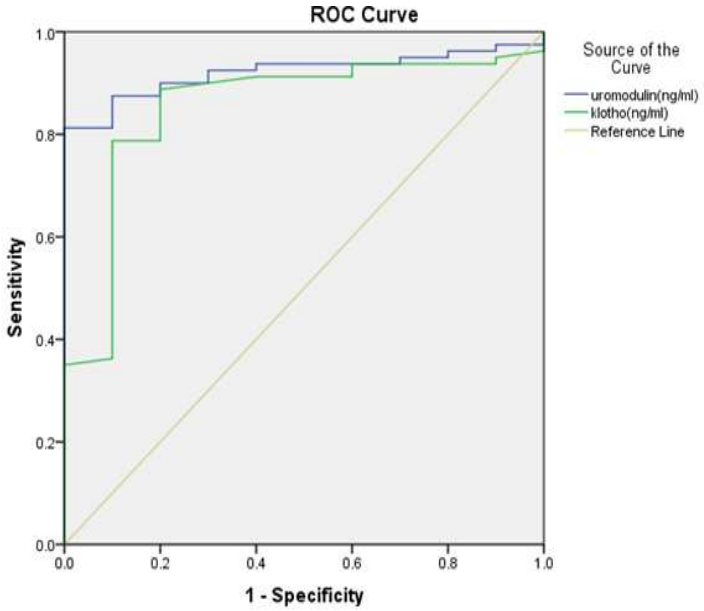

Figure 1: Receiver Operating Characteristic (ROC) curves analysis the diagnostic performance of serum Uromodulin and serum α-Klotho in relation to eGFR.

Table 3 shows significant alterations in iron metabolism parameters across CKD stages. Mean serum iron concentrations decreased progressively from 82.87 ± 14.96 µg/dL in Stage 1 to 25.95 ± 7.37 µg/dL in Stage 5 ($p < 0.001$). Similarly, TIBC declined from 283.47 ± 62.47 µg/mL to 186.48 ± 54.12 µg/mL across the same stages ($p < 0.001$).In contrast, serum ferritin levels increased progressively with worsening CKD severity, rising from 198.25 ± 46.21 ng/mL in Stage 1 to 724.63 ± 219.11 ng/mL in Stage 5 ($p < 0.001$).These findings are consistent with disturbances in iron homeostasis commonly observed in advanced CKD; however, the present study was not designed to determine underlying mechanisms or causal relationships.

Table 3: Mean Levels of Iron Metabolism Markers in Different Stages of CKD.

Variables	Stage 1 (n=4)	Stage 2 (n=10)	Stage 3a (n=5)	Stage 3b (n=13)	Stage 4 (n=20)	Stage 5 (n=38)	p value
Iron (µg/dl)	82.87±14.96	51.86±12.14	48.82±4.81	40.24±8.64	34.39±6.43	25.95±7.37	<0.001*
Ferritin (ng/ml)	198.25±46.21	258.05±101.29	343.38±101.68	376.39±56.56	405.65±58.05	724.63±219.11	<0.001*
TIBC (µg/ml)	283.47±62.47	228.82±22.30	220.48±7.37	199.13±13.04	193.63±35.88	186.48±54.12	<0.001*

*Statistically significance

TIBC- Total iron Binding Capacity

DISCUSSION: In our study, out of 90 patients, 61% were male and 38.9% were female have CKD. In contrast with our study, Michal Lewandowski et al. investigated that the higher CKD prevalence is in women[19]. For stages 3–5, the frequency of CKD was higher in women than in men. The eGFR calculations adjust for sex to account for variations between men and women. For example, increased muscle mass in male results in a higher creatinine level and a lower eGFR. Both sex evaluations of the eGFR formulas revealed no sex-specific variations in estimating bias[20]

Aligning with our results, Litvin et al. stated that chronic kidney diseases (CKDs) are characterized by impaired kidney function and structure lasting over three months. The likelihood of developing CKD rises with advancing age. Additionally, CKD significantly increases the risk of cardiovascular illness and death[21].

In comparison to over-studying, according to Levey AS and Coresh J investigated a decreasing eGFR indicates ongoing nephron damage and reduced kidney function, making it valuable for both diagnosis and prognosis. As renal function deteriorates, eGFR becomes more reliable in forecasting negative outcomes such as cardiovascular complications, death, and progression to end-stage kidney disease (ESKD)[22].

Our study was carried out on serum markers (Uromodulin and α-klotho). The results of the current study indicate that serum Uromodulin and α-klotho levels were significantly decreased in patients with CKD. There was a significant difference ($p < 0.001$) in all groups of CKD patients. Dominic Stebl et al. found that serum uromodulin levels are reduced in individuals with chronic kidney disease (CKD). They reported that people without CKD had significantly higher serum uromodulin concentrations compared to those with CKD at any stage[23].

Risch et al. reported that serum uromodulin levels declined as kidney function worsened. Their study also demonstrated that serum uromodulin was negatively associated with creatinine levels and positively correlated with eGFR, consistent with the findings of the current study[24]. Pracjczer et al. found a positive correlation between eGFR and serum uromodulin levels, aligning with the results of the current study. They suggest that uromodulin may play an active role in the progression of chronic kidney disease (CKD)[5].Lv L. et al. concluded that reduced serum uromodulin levels were independently linked to a decline in kidney function[25].

Seibert et al. found that serum α-Klotho levels in patients gradually declined across CKD stages 1 to 5. Another study using immunohistochemical staining to assess renal α-Klotho expression revealed a significant decrease in kidney α-Klotho levels corresponding to AKI severity[26]. Erben RG and Andrukhova O reported that serum α-Klotho levels were significantly elevated in CKD patients compared to healthy controls[27] . Supporting our findings, Lim et al. reported that serum α-Klotho levels are initially reduced in patients with CKD. Additionally, we observed a positive correlation between serum α-Klotho and vascular calcification[28]. In line with our findings, Hu et al. reported that serum α-Klotho could serve as a straightforward, quick, and early biomarker for CKD[29].

Mittal A et al. reported comparable findings in a study showing a significant rise in mean serum creatinine and urea levels in CKD patient[30]. This is consistent with the study by Rani BS et al. who found that the median serum urea and creatinine level was higher in CKD patients[31]. Similar findings were reported by Onyango A et al. which showed higher levels of serum urea and creatinine in chronic kidney disease[32].

Talwar et al. However, the mean serum ferritin levels appear elevated due to an uneven distribution of values. This elevation is also attributed to the fact that the majority of cases in our study involved anaemia of chronic disease, which is associated with lower mean TIBC levels[33]. In contrast to our findings, Singh et al. observed lower serum ferritin levels and higher mean serum iron levels. This discrepancy can be linked to the patients' usage of EPO medication, as the decrease in serum ferritin indicates that the erythroid cells proliferation is utilizing iron. However, EPO was only administered to a limited percentage of patients in our trial, which accounts for the lack of a discernible drop in mean blood ferritin levels[34].

Our ROC analysis demonstrated that serum uromodulin and α-Klotho were capable of distinguishing advanced CKD from earlier stages of disease, with serum uromodulin showing superior performance. However, because the study included only patients with established CKD and relatively few participants in the early stages of disease, these findings should not be interpreted as evidence for population screening or early detection of CKD. Prospective studies including healthy controls and larger numbers of early-stage CKD patients are required to evaluate their utility as early diagnostic biomarkers.

Limitations of the study:

The cross-sectional design precludes assessment of causality, disease progression, or prediction of renal outcomes.

- The study was conducted at a single centre, potentially limiting generalisability.
- The sample size was relatively small and unevenly distributed across CKD stages, with a predominance of advanced CKD.
- Potential confounding factors, including comorbidities, medications, and duration of CKD, were not comprehensively evaluated.

Conclusion:

Serum uromodulin and α-Klotho levels were significantly associated with CKD severity and renal function in this cross-sectional cohort. Both biomarkers

declined progressively with worsening CKD stage and demonstrated significant correlations with eGFR. Serum uromodulin showed stronger discriminatory performance than α-Klotho in differentiating advanced CKD from earlier stages. However, because of the study's single-centre cross-sectional design, limited sample size, and predominance of advanced CKD stages, the findings should be regarded as exploratory. Larger prospective multicentre studies are required to determine whether these biomarkers provide clinically meaningful information beyond conventional measures of kidney function and whether they can predict CKD progression or adverse renal outcomes.
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REFERENCES:

1. Kamal A. Estimation of blood urea (BUN) and serum creatinine level in patients with renal disorder. *Indian J FundamAppl Life Sci.* 2014;4:199–202.
2. Dorgelo A, Oostrom TAJ. An integrated approach towards a public health perspective on chronic kidney disease. *Nat Rev Nephrol.* 2022;18(3):131–132.
3. Burtis CA, Ashwood ER, Bruns DE. *Tietz textbook of clinical chemistry and molecular diagnostics* [e-book]. 5th ed. Philadelphia: Elsevier Health Sciences; 2012.
4. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (Tamm–Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. *Kidney Int.* 2011;80(4):338–347.
5. Prajczer S, Heidenreich U, Pfaller W, Schutz GJ, Romagnani P, Stowasser M, et al. Evidence for a role of uromodulin in chronic kidney disease progression. *Nephrol Dial Transplant.* 2010;25(6):1896–1903.
6. Mian IS. Sequence, structural, functional, and phylogenetic analyses of three glycosidase families. *Blood Cells Mol Dis.* 1998;24(2):83–100.
7. Bi X, Yang K, Zhang B, Zhao J. The protective role of Klotho in CKD-associated cardiovascular disease. *Kidney Dis (Basel).* 2020;6(6):395–406.
8. Hu MC, Kuro-o M, Moe OW. Klotho and kidney disease. *J Nephrol.* 2010;23(Suppl 16):S136–S144.
9. Manoeuvrier G, Bach-Ngohou K, Batard E, Masson D, Trewick D. Diagnostic performance of serum blood urea nitrogen to creatinine ratio for distinguishing prerenal from intrinsic acute kidney injury in the emergency department. *BMC Nephrol.* 2017;18:173.
10. National Kidney Foundation. Facts about chronic kidney disease. 2020. Available from: https://www.kidney.org/atoz/content/about-chronic-kidney-disease. Accessed 2024 Feb 8.
11. Matovinović MS. Pathophysiology and classification of kidney diseases. *EJIFCC.* 2009;20:2.
12. Thang LV, Kien NT, Van Hung N, Kien TQ, Dung NH, Thu Huong NT, et al. Serum total iron-binding capacity and iron status in patients with non-dialysis-dependent chronic kidney disease: a cross-sectional study in Vietnam. *Asia Pac J ClinNutr.* 2020;29(1):48–54.
13. Moranne O, Froissart M, Rossert J, Gauci C, Boffa JJ, Haymann JP, et al. Timing of onset of CKD-related metabolic complications. *J Am SocNephrol.* 2009;20:164–171.
14. Regidor D, McClellan WM, Kewalramani R, Sharma A, Bradbury BD. Changes in erythropoiesis-stimulating agent dosing and haemoglobin levels in US non-dialysis chronic kidney disease patients between 2005 and 2009. *Nephrol Dial Transplant.* 2011;26:1583–1591.
15. Babitt JL, Lin HY. Mechanisms of anemia in CKD. *J Am SocNephrol.* 2012;23:1631–1634.
16. Mehdi U, Toto RD. Anaemia, diabetes, and chronic kidney disease. *Diabetes Care.* 2009;32(7):1320–1326.
17. Iyawe IO, Adejumo OA, Iyawe LI, Oviasu EO. Assessment of iron status in predialysis chronic kidney disease patients in a Nigerian tertiary hospital. *Saudi J Kidney Dis Transpl.* 2018;29:1431–1440.
18. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis.* 2014;63(5):713–735.
19. Lewandowski MJ, Simon K, Amelie K, Philipp B, Martina S, Elisabeth S, et al. Chronic kidney disease is more prevalent among women but more men than women are under nephrological care. *Cent Eur J Med.* 2023;135:89–96.
20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604–612.
21. Litvin CB, Hyer JM, Ornstein SM. Use of clinical decision support to improve primary care identification and management of chronic kidney disease. *J Am Board Fam Med.* 2016;29:604–612.
22. Levey AS, Coresh J. Chronic kidney disease. *Lancet.* 2012;379(9811):165–180.
23. Steubl D, Banas MC, Hettwer K, Anders HJ, Wolf G, Schmaderer C, et al. Plasma uromodulin correlates with kidney function and identifies early stages in chronic kidney disease patients. *Medicine (Baltimore).* 2016;95(10):e3011.
24. Risch L, Lhotta K, Meier D, Medina-Escobar P, Nydegger UE, Risch M. The serum uromodulin

level is associated with kidney function. *ClinChem Lab Med.* 2014;52(12):1755–1761.
25. Lv L, Wang J, Gao B, Wu L, Wang F, Cui Z et al. Serum uromodulin and progression of kidney disease in patients with chronic kidney disease. J Transl Med. 2018 Nov 19;16(1):316.
26. Seibert E, Radler D, Ulrich C, Hanika S, Fiedler R, Girndt M. Serum klotho levels in acute kidney injury. *ClinNephrol.* 2017;87(4):173–179.
27. Erben RG, Andrukhova O. FGF23–Klotho signaling axis in the kidney. *Bone.* 2017;100:62–68.
28. Lim K, Lu TS, Molostvov G, Lee C, Lam FT. Vascular klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation.* 2012;125:2243–2255.
29. Hu MC, Kuro-o M, Moe OW. The emerging role of klotho in clinical nephrology. *Nephrol Dial Transplant.* 2012;27:2650–2657.
30. Mittal A, Sathian B, Kumar A, Chandrasekharan N, Sunka A. Diabetes mellitus as a potential risk factor for renal diseases among Nepalese: a hospital-based case–control study. *NJE.* 2010;1(1):22–25.
31. Rani BS, Suchitra MM, Rao PS, Kumar VS. Serum tumor markers in advanced stages of chronic kidney disease. *Saudi J Kidney Dis Transpl.* 2019;30(4):898–904.
32. Onyango A, McLigeyo S, Mwango A, Otieno D, Ngigi J, Chemutai P, et al. Serum urea, electrolytes, creatinine and estimated glomerular filtration rates in patients with chronic kidney disease at initiation of haemodialysis. *Kidney Int Rep.* 2023;8(3 Suppl):S237.
33. Talwar VK, Gupta HL, Narayan S. Clinicohaematological profile in chronic renal failure. *J Assoc Physicians India.* 2002;50:228–233.
34. Singh NP, Aggarwal L, Singh T, Anuradha S, Kohli R. Anaemia, iron studies and erythropoietin in patients with chronic renal failure. *J Assoc Physicians India.* 1999;47:284–290.