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Research Article

Biomarkers for Chronic Kidney Disease

Comparison of Biomarkers to Differentiate Chronic Kidney Disease and Chronic Kidney Disease of Unknown Etiology

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Abstract: Chronic kidney disease of unknown etiology (CKDU) is prevalent and clinically silent until its late stages at which patient may suffer significant irreversible damage, or mortality in the absence of early screening and intervention. CKDU is asymptomatic but may show nonspecific symptoms. Conventional markers are influenced by multiple non-renal factors while Kidney injury molecule-1(KIM-1) and Neutrophil gelatinase-associated lipocalin(NGAL) are promising biomarkers of chronic kidney disease (CKD), there are no studies reported so far on these markers in CKDU. The aim of the study is to assess the sensitivity of biomarkers serum KIM-1 and NGAL in patients with CKDU in comparison with CKD and controls. To achieve this, our objectives are to estimate the level of biochemical parameters like serum creatinine, urea, uric acid, random blood sugar, systolic and diastolic blood pressure and hemoglobin and to compare novel biomarkers KIM-1 and NGAL in CKDU patients with CKD. The serum levels of urea, uric acid, creatinine, KIM-1 and NGAL were estimated in control, CKD and CKDU (n = 35, 46, 79 respectively). Creatinine showed 8.1-fold and 2.7-fold increase (P <0.001), and urea 4.0% and 1.8% increase (P < 0.001), compared to control in CKD and CKDU cohorts, respectively. The sensitivity and specificity of KIM-1 was 73.4% and 67.4%with a cutoff value of 203.5ng/mL using the receiver operating characteristic(ROC) curve. For NGAL they were 82.3%, 73.9% and 255ng/mL. Compared to control, KIM-1 showed a 51.5-fold and 87.2-fold increase in CKD and CKDU, respectively (P < 0.001). NGAL showed a 4.8-fold and 6.7-fold increase in CKD and CKDU, respectively (P < 0.001). Creatinine and urea were higher in CKD than in CKDU, whereas KIM-1 and NGAL were higher in CKDU than CKD. KIM-1 and NGAL are sensitive biomarkers for CKDU. KIM-1 can differentiate CKD and CKDU.

Keywords: Biomarkers; Chronic kidney disease of unknown etiology; Kidney injury molecule-1; Neutrophil gelatinase-associated lipocalin.

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1. INTRODUCTION

Chronic kidney disease (CKD) is one of the major problems of human health, globally. Diabetes and hypertension are the leading causes of CKD.[1] The Global burden of disease (GBD) study 2017 ranked chronic kidney disease 12th among the causes of death globally and its prevalence was 9.1%. In many countries, chronic kidney disease is now among the top five causes of death.[2] Glomerulonephritis and tubulointerstitial disease of unknown etiology are also prevalent in Asia and Sub Saharan Africa. It is described as CKD of unknown etiology (CKDU).[3] There are several global epidemics of unexplained kidney disease, based on geographical divergence e.g., Balkan endemic nephropathy(BEN), Meso American nephropathy(MeN), Itai-Itai disease in Japan, Sri Lankan agricultural nephropathy and Uddanam endemic nephropathy in India.[4,5] Such types of nephropathies are typically reported from warm, low altitude coastal, subcostal, tropical and subtropical regions of the world.[5] The disease pattern is similar in characteristics of late presentation, long asymptomatic phase, minimal or no proteinuria and nonappearance of hypertension in early phases of the disease without reduction in glomerular filtration rate (GFR).[4] Kidney biopsy has revealed tubular atrophy and interstitial fibrosis with inflammatory cells.[6] It is fatal due to late recognition and rapid disease progression.[7] In Uddanam region, Southern Indian state of Andhra Pradesh prevalence of CKD was 18.23%. 73% of patients with CKD in Uddanam were identified as CKDU.[7] In India, CKDU is present in younger and poorer people.[3] In the early stages, the symptoms of CKDU are usually not apparent. A significant reduction in kidney functions is obvious at the later stage of the disease. If diagnosed early (stage 1-3), the progression of CKDU can be reduced and complications can be minimized. In stages 4 and 5, kidney damage would be extensive with peak levels of urea and creatinine.[8] The diagnosis and staging of CKD rely on the measurement of GFR and albuminuria.[6] The actual GFR by measurement of external filtration markers is tedious, cumbersome and may not be practical.[3] Therefore, the clinical assessment depends on the levels of urea and creatinine. However, there are shortcomings with creatinine levels because of the low predictive value.[9] Hence, next-generation biomarkers for early diagnosis of CKDU are very much essential and should not be influenced by age, nutritional status, or concurrent health concerns. It should provide rapid non-invasive and specific measurements correlating with kidney tissue pathology. To our knowledge, this is first cross-sectional study to show the relationship between novel serum biomarkers kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in CKDU in Uddanam nephropathy, India. Previous cross-sectional studies have shown that urinary and serum KIM-1 and NGAL were identified as early biomarkers of acute kidney injury(AKI).[10] NGAL role was studied in SLE, CKD stages 2-4, IgA nephropathy, glomerulonephritis, Autosomal dominant polycystic CKD,[10] Drug-induced chronic tubulointerstitial nephritis.[10,11] Urinary KIM-1 studied in proteinuric non-diabetic CKD, in type1 Diabetes mellitus patients.[12] Earlier works on KIM-1 and NGAL did not include all stages of CKD.[13] Reports were not available on serum levels of KIM-1 and NGAL in CKDU of Uddanam nephropathy compared with CKD. Hence, there is a need for biomarkers that diagnose CKDU early. The aim of the present study was to assess serum KIM-1 and NGAL as biomarkers in patients with CKDU in comparison with CKD and to estimate the specificity and sensitivity of these markers.

2. MATERIALS AND METHODS

2.1. Studydesign

A comparative, cross-sectional study was conducted in the out-patient section of the Department of General Medicine, Government General Hospital and Medical College (Srikakulam, Andhra Pradesh, India) from April to September2019. A total of 160 participants were involved, 79 with CKDU and 46 with CKD. For comparison, 35 healthy controls who visited for general check-ups were also included. Ethical approval was taken from the Institutional Ethics Committee (Government Medical College, Srikakulam) with reference no: ECR/492/inst/AP/2013/RR-16. Written informed consent was taken from all the participants. The inclusion criteria for the study were both genders with ages between 18 to 65 years. CKD stages were defined by National Kidney Foundation, USA, under Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines.[14] CKD is defined as the presence of either kidney damage as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies or glomerular filtration rate (GFR)<60mL/min/1.73m^2 for ≥ 3 months.[14] Serum creatinine >1.2mg/dL was taken as the indicator of kidney damage.[6] GFR was estimated using predictive equations.[6,14]

Inclusion

Patients of both genders willing to participate above 18 years, serum creatinine >1.2mg/dL, blood pressure systolic/diastolic<140/90mmHg, random blood sugar <200 mg/dL were taken as CKDU and others with diabetes mellitus, hypertension and serum creatinine >1.2 mg/dL were taken as CKD.[6]

Exclusion

Age below 18 years and above 65 years, Pregnant and lactating mothers, Chronic systemic illnesses and patients who are not willing to give informed consent were excluded from the study.

2.2 Biochemical analysis

From the antecubital vein 5mL of blood was taken aseptically and collected in polypropylene tubes. The blood was allowed to clot in an upright position for 30 min before centrifugation. The samples were centrifuged at 3000 rpm for 10 min and serum was separated and used for the analysis of creatinine, urea, uric acid, KIM-1 and NGAL.All chemicals and reagents used in this study were of analytical grade.Creatinine was measured by Jaffe's kinetic method[15], urea by urease method[16] and uric by enzymatic method.[17] Serum KIM-1 and NGAL kits were purchased from KINESIS Dx, (Los Angeles, California). Serum KIM-1 and NGAL levels were analyzed by enzyme-linked immunosorbent assay (ELISA). The kit employs a double-antibody sandwich ELISA to estimate the levels of serum KIM-1 and NGAL in samples.

2.2.1 Kidney injury molecule-1 (KIM-1) assay procedure

Human KIM-1 was measured in serum sample using ELISA (KINESIS Dx, Los Angeles, California Cat No. K12-1100) according to the manufacturer's instructions. Kim-1 ELISA kit employs quantitative double antibody sandwich enzyme immunoassay technique. The detection range of the kit was

(0.4-6.4ng/mL). Absorbance was measured at 450 nm. Standard solutions were prepared using standard concentration and diluent provided in the kit according to manufacturer's instructions. 50µl KIM-1 standard and 40µl test solution were added to the respective wells pre-coated with monoclonal human KIM-1 antibody. These were incubated for 10 minutes at 37 ^{0}c. 10µl of KIM-1 Ab biotin conjugate was added followed by 50µl of HRP conjugate to form an immune complex and incubated for 1 hour. Plates were washed with a wash buffer for removal of the unbound immune complex. Substrate (chromogenic solution) A and B 50µl each were added till the blue color turns to yellow. Performance was read at 450 nm within 15 minutes after adding the stop solution. Results were calculated by drawing a standard curve by taking density on X-axis, and OD values on Y-axis.[18]

2.2.2 Neutrophil gelatinase-associated lipocalin (NGAL) assay procedure

Human NGAL was measured in serum sample using ELISA (KINESIS Dx, Los Angeles, California Cat No. K12-1720) according to manufacturer's instructions. NGAL ELISA kit employs quantitative double antibody sandwich enzyme immunoassay technique. The detection range of the kit was (80-200ng/mL). Absorbance was measured at 450 nm. Standard solutions were prepared using standard concentration and diluent provided in kit according to the manufacturer's instructions. 100µl NGAL standard and 40µl test solution were added to the respective wells pre-coated with monoclonal human NGAL antibodies. These were incubated for 10 minutes at 37 ^{0}c. 20µl of NGAL Ab biotin conjugate was added followed by 100µl of HRP conjugate to form an immune complex and incubated for 1 hour. Plates were washed with a wash buffer for removal of the unbound immune complex. Substrates A and B 100µl each were added till the blue color turns to yellow. Performance was read at 450 nm within 15 minutes after adding stop solution. Results were calculated by drawing a standard curve by taking density on X-axis, OD values on Y-axis.[18]

2.3. Demographic and clinical variables

The demographic and clinical variables were obtained through a questionnaire. Height, weight, and systolic and diastolic blood pressures were measured by standard methods.

2.4 STATISTICAL ANALYSIS

SigmaPlot 14.5 version (Systat Software Inc, USA) was used for sample size calculation and for the analysis of data. The sample size was estimated for a 25% difference among the three groups, with 30% of standard deviation, 90% power and 5% significance level. The estimated minimum sample size was 40 for each group. However, during the study period of 6 months, all the CKD and CKDU with the control were taken (CKD=46, CKDU=79 and control=35). The significance of difference in the means between groups was tested with one-way ANOVA with Bonferroni 't' test. The receiver operating characteristics (ROC) curves, and the analysis were carried out for creatinine, KIM-1 and NGAL of CKD and CKDU (excluding control), using SigmaPlot 14.5 version (Systat Software Inc, USA) for sensitivity, specificity, area under the curve with confidence interval and cut off values. From the ROC curve analysis, the sensitivity and specificity were taken, where the values were very close. A probability of 0.05 and less was considered as statistically significant.

3. RESULTS

The demographic and clinical variables of CKD and CKDU were given in Table 1. Many significant differences were observed between the two categories. Though CKDU is present in males and females in equal proportion, CKD is shown in 93.5% of males ($P < 0.025$). Among the age groups, CKDU affects even younger people.22.8% was affected by CKDU, while only 4.3% were by CKD ($P < 0.001$). CKDU is common in less educated people and many of them are fishermen ($P < 0.001$). One-third of individuals with CKDU had a family history of kidney disease (P=0.041). Though, alcohol consumption does not significantly contribute to kidney disease ($P = 0.082$), smoking causes more CKDU ($P = 0.26$). Higher BMI of overweight and obesity (> 25) shows more CKD (76.1%) than CKDU (32.9%) (P<0.001). This is due to higher weight individuals in the CKD group ($P < 0.001$).

Table 1: Demographic variables of chronic kidney disease (CKD) and chronic kidney disease of unknown etiology (CKDU).

Characteristic	Category	CKD	CKDU	Statistics χ^2	P-value
Gender	Male	43	41	20.956	**0.025**
	Female	3	38		
Age, year	<30	2	18		
	31-50	26	35	7.385	**< 0.001**
	>51	18	26		
Education	School level	32	79	24.102	**< 0.001**
	Graduates	14	0		
Family history of CKD	Yes	4	20	4.161	**0.041**
	No	42	59		
Smoking habit	Yes	11	36	4.925	**0.026**
	No	35	43		
Alcohol habit	Yes	18	18	3.033	0.082
	No	28	61		
Height, cm	< 160	16	39		
	161 – 170	14	36	19.119	**< 0.001**
	> 171	16	4		

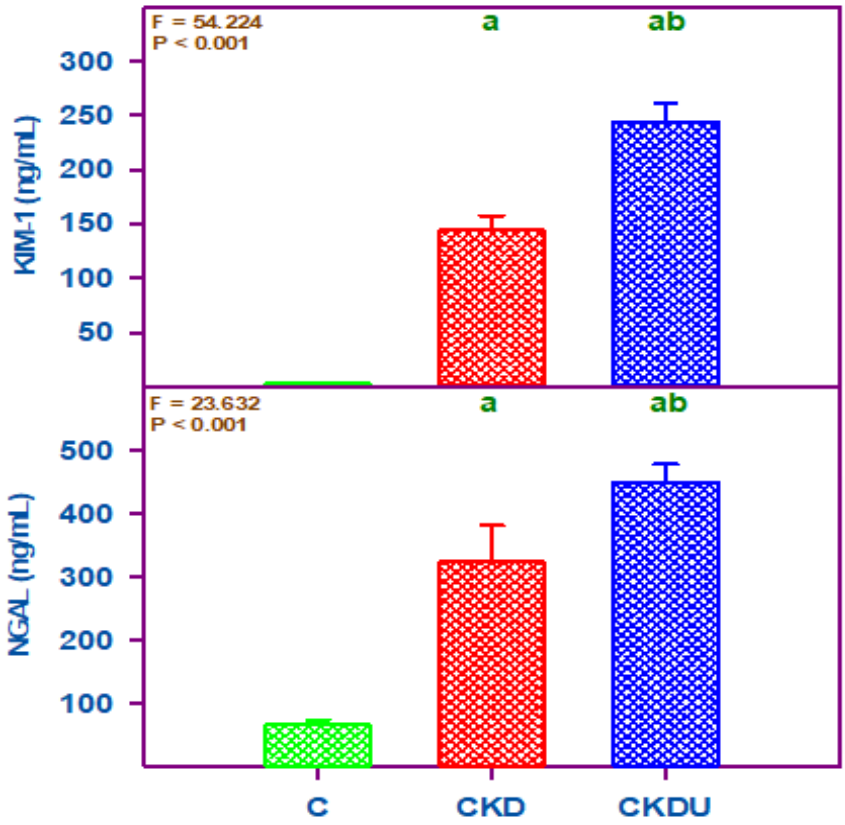
Weight, kg	< 60	6	50	31.226	**< 0.001**
	61 – 70	12	5		
	> 71	28	24		
BMI, kg/m^2	< 24.9	11	53	21.807	**< 0.001**
	25.0 – 29.9	33	25		
	> 30.0	2	1		

Table 1 illustrates the significant difference among CKDU and CKD with respect to demographic and clinical variables. CKDU affected younger and less educated people who usually do strenuous work in a hot climate for a longer period. The mean, standard error and statistical information were given in Table 2. The serum uric acid and random blood sugar levels did not show any statistical significance among the three groups (P=0.254 and P = 0.051respectively). The hemoglobin level showed 7.8% and 8.8% decrease in CKD and CKDU in comparison to control (P=0.019). Compared to the control group CKD and CKDU, showed 27.6% and 1.1% increase in systolic blood pressure (P <0.001), and 14.2% and 0.5% increase in diastolic blood pressure (P <0.001). Serum creatinine showed 8.1 fold and 2.7-fold increase (P <0.001), and serum urea 4.0% and 1.8% increase (P < 0.001), compared to control groups in CKD and CKDU.

Table 2: Comparison of mean values of different parameters between control, CKD and CKDU.

Parameter	Group	Mean	SEM	Statistics F	P value
Serum Creatinine (mg/dL)	C	0.914	0.028	133.22	**<0.001**
	CKD	7.394[a]	0.47		
	CKDU	2.434[ab]	0.152		
Serum Urea (mg/dL)	C	25.5	2.6	77.877	**<0.001**
	CKD	102.6[a]	5.5		
	CKDU	46.0[ab]	3.4		
Serum Uric Acid (mg/dL)	C	4.315	0.086	1.384	0.254
	CKD	4.535	0.135		
	CKDU	4.375	0.053		
Haemoglobin (g/dL)	C	10.2	0.3	4.054	**0.019**
	CKD	9.4	0.3		
	CKDU	9.3[a]	0.2		
Random blood sugar (mg/dL)	C	106.9	3.1	3.036	0.051
	CKD	115.7	4.0		
	CKDU	106.2	2.1		
Systolic blood pressure(mmHg)	C	120.3	1.6	62.573	**<0.001**
	CKD	153.5[a]	4.0		
	CKDU	121.6[b]	1.1		
Diastolic blood pressure(mmHg)	C	78.1	1.4	26.832	**<0.001**
	CKD	89.2[a]	1.8		
	CKDU	77.7[b]	0.8		

Table 2 illustrates the estimation and comparison of mean values of biochemical parameters between CKDU, CKD and controls. The serum creatinine, urea, haemoglobin systolic and diastolic blood pressure in CKDU patients showed significant difference from CKD and control groups. The mean ± SEM of serum KIM-1 of control, CKD and CKDU were 2.8 ± 0.3, 144.2 ± 13.7 and 244.2 ± 16.6(ng/mL) respectively. Compared to the control group, serum KIM-1 showed 51.5 fold and 87.2-fold increase in CKD and CKDU respectively. The mean ± SEM of serum NGAL of control, CKD and CKDU were 66.8±8.0, 323.3±58.3 and 450.1±27.7(ng/mL) respectively. Compared to the control group, serum NGAL showed 4.8 fold and 6.7-fold increase in CKD and CKDU, respectively (Figure 1). The increase of serum creatinine and urea was higher in CKD than in CKDU, whereas KIM-1 and NGAL were higher in CKDU than CKD.



Values are mean ± SE (n = C = 35; CKD = 46; CKDU = 79).
The 'F' and 'P' values are by one way ANOVA with the Bonferroni 't-test.
[a]Significantly different from the control group.
[b]Significantly different from the CKD group.

Fig 1. Kidney injury molecule-1 (KIM-1) and Neutrophil gelatinase-associated lipocalin (NGAL) in control (c), chronic kidney disease (CKD) and chronic kidney diseaseof unknown etiology (CKDU).

Figure 1 illustrates that serum KIM-1 and NGAL showed a significant fold increase in CKDU than CKD whereas increased levels of serum creatinine and urea were higher in CKD than CKDU. The receiver operating characteristic (ROC) curves were used to determine the clinical accuracy to the target markers, KIM-1 and NGAL, compared with creatinine, for CKD and CKDU. ROC plots were constructed and the area under the curve (AUC) with 95% confidence interval, sensitivity, and specificity was calculated (Figure 2). The area under ROC curve for serum creatinine was 0.12 (P=1.0), for KIM-1 = 0.74 (P < 0.001) and for NGAL = 0.82 (P < 0.001), showing that creatinine cannot differentiate CKD and CKDU, whereas KIM-1 and NGAL can differentiate CKD and CKDU. The cut off value for KIM-1 was 203.5 ng/mL, with a sensitivity and specificity of 73.4% (CI = 62.3% - 82.7%) and 67.4% (52.0% - 80.5%), respectively. The cut off value for NGAL was 255.0 ng/mL, with a sensitivity and specificity of 82.3% (CI = 72.1% - 90.0%) and 73.9% (58.9% - 85.7%), respectively. The histogram in Figure 2, shows the distribution of creatinine, KIM-1 and NGAL in CKD and CKDU. There were four extreme values of NGAL in CKD and one in CKDU (>1000 ng/mL). Whereas, the values of KIM-1 were closely distributed with less variation. Figure 2 illustrates that the ROC curve used to calculate the accuracy of biomarkers and AUC, sensitivity and specificity of each biomarker was calculated and compared.

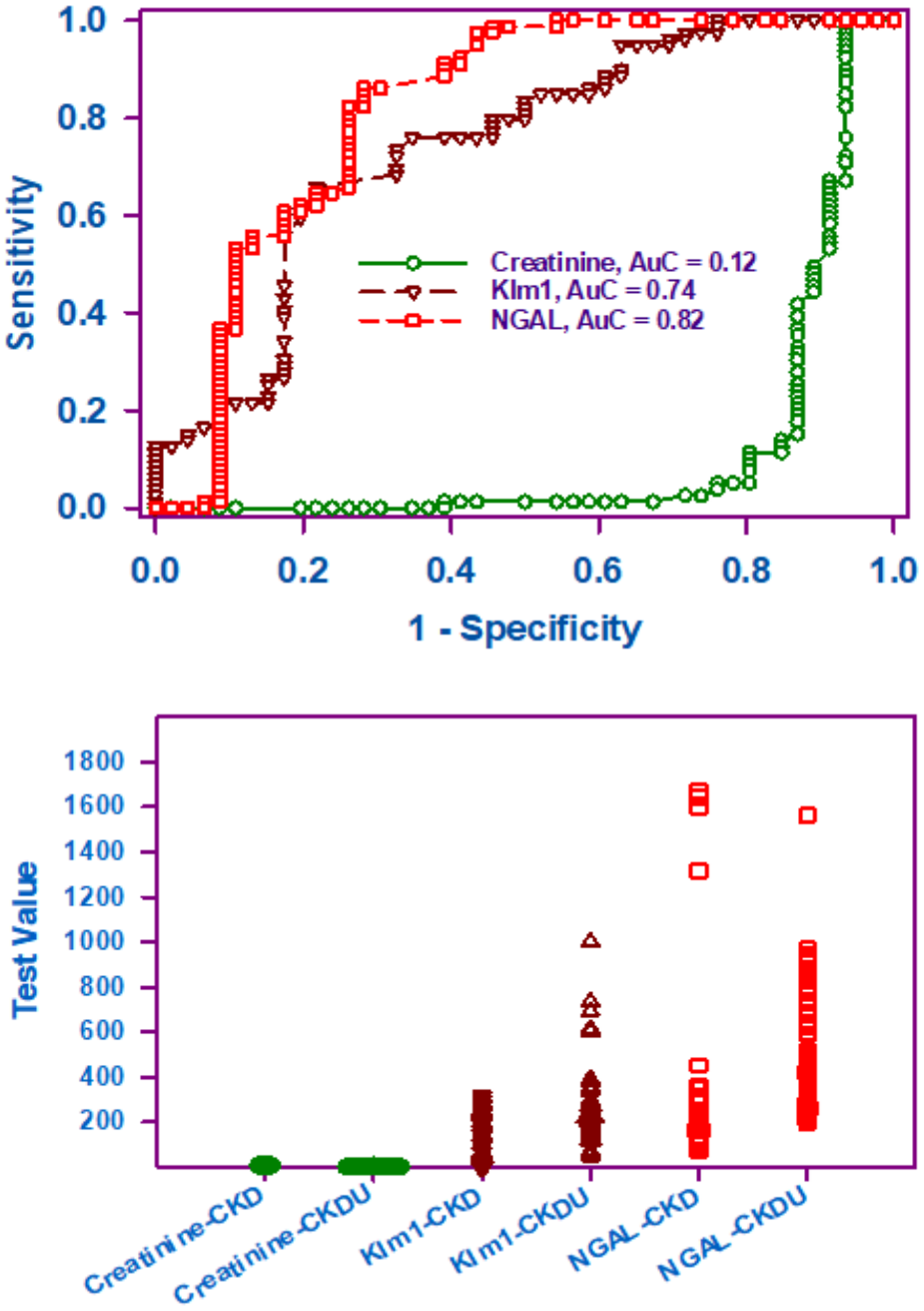


Fig 2: Receiver operating characteristic (ROC) curve and histogram of serum creatinine, KIM-1 and NGAL of CKD and CKDU.

4. DISCUSSION

The present study shows that CKDU affects younger age groups also unlike CKD. Most of the individuals of CKDU were less educated, and either fishermen or agricultural workers. These individuals are continuously exposed to difficult environmental conditions and may also be exposed to various chemicals. Exposure to heat for a longer time with physical exertion may cause dehydration. Recurrent dehydration may lead to low grade renal injury. Chronic kidney disease is accepted as leading cause of CKDU in Central America.[19] Dehydration causes release of vasopressin, increase in serum osmolarity and also activation of aldose reductase in renal medulla causing tubular damage.[20] The assessment of normal kidney function is based on the measurement of serum creatinine and urea levels. Significant increase in the levels of serum creatinine and urea indicate renal tubule damage and is a sign of CKD.[7] However, these markers have been shown to be lacking a high predictive value.[8,10] In the present study, a significant elevation in serum creatinine was seen in both CKD and CKDU patients. A cross sectional study conducted in Sri Lanka among agriculture communities in CKDU[18] and another study conducted in South Korea in acute kidney injury patients with scrub typhus[21] showed elevated levels of serum creatinine. In the present study, CKD showed higher levels of creatinine than CKDU due to direct involvement of the kidney function. Creatinine is a metabolite from the nonenzymatic hydrolysis of creatine and phosphocreatine, the two substances which are found almost exclusively in muscle[22] as well as obtained from dietary intake.[23] Creatinine does not bind to plasma proteins. It is filtered by glomerulus, secreted by the tubules and not reabsorbed. Therefore, the clearance can be measured as an indicator of GFR.[22] Serum or plasma creatinine concentration not only depend on GFR but also on muscle mass and dietary protein intake.[22] Factors such as change in body composition due to age, gender, race and physical activity can affect creatinine levels.[24] Availability of several assay procedures

makes creatinine an endogenous filtration marker.[22] It was reported that proteins, glucose, ascorbic acid, creatine, ketone bodies, pyruvate, guanidine and cephalosporins interfere with creatinine values.[25] Although, creatinine value is widely used as a marker of renal function, it is unstable and should be measured without delay. In cases of severe renal dysfunction, the active tubular secretion of creatinine will account for overestimation of GFR.[22] It is also influenced by nonrenal variables. In addition, creatinine is contained in intestinal secretions and can be degraded by bacteria. If GFR is reduced, the amount of creatinine elimination through this extra-renal route is increased.[23] It is insensitive to even significant declines in GFR due to the nonlinear relationship between creatinine and GFR. Creatinine concentrations increase in serum when 40-50% of renal parenchyma is reversibly or irreversibly damaged.[8] This is due to compensatory hyperfiltration of remaining functioning nephrons, secretion of creatinine and extra-renal elimination of creatinine, as GFR declines.[24] This may lead to a lack of detection in the early stages of chronic kidney failure. It is a poor screening tool for early kidney disease. In the present study, CKDU showed a minimal increase in creatinine. The present work shows significantly elevated serum urea levels in both CKD and CKDU when compared with control. An earlier study also revealed an elevation of blood urea levels in CKD.[27] Urea is the primary metabolite derived from dietary protein and tissue protein turnover. It is freely filtered at the glomerulus but not secreted.[26] It is reabsorbed by the renal tubules. In addition, as the urine flow rate decreases, more urea is reabsorbed. Serum urea levels are inversely correlated with the decline of kidney function and are also affected by extra-renal factors such as protein intake, gastrointestinal bleeding, catabolic states, malnutrition, heart failure, dehydration, use of glucocorticoids, and hepatic urea synthesis.[26] In CKD, the declining kidney function, is characterized by elevation of blood urea nitrogen. This may promote the formation of isocyanate. Urea slowly dissociates into cyanate, which is rapidly converted to isocyanate. Isocyanate is a reactive electrophile with a high affinity for nucleophilic groups such as primary amines and is highly toxic.[27] In the present study, no significant change in serum uric acid, and random blood sugar occurred in all the study groups. In a cohort study in the Japanese population, subjects with threshold serum uric acid (SUA) ≥ 6.0mg/dL with a hazard ratio 1.22, 95% CI (0.07-1.390) had a significantly increased risk for CKD and a rapid decline in eGFR compared with subjects with SUA of 4-4.49mg/dL.[28] In a prospective study no significance could be identified for blood sugar and also haemoglobin.[29] In the present study hemoglobin showed a low levels in CKDU and CKD. The blood pressure assessment indicates that CKD shows significantly elevated levels of SBP and DBP than the control group. The present study findings are not consistent with previous case-control studies that dealt with heavy metals and pesticides in chronic kidney disease.[30] Cys-C is another widely used biomarker in GFR estimation independent of gender, age or muscle mass.[20] But, it is influenced by body mass, abnormal thyroid function, corticosteroids and systemic inflammation.[31,32] Proteinuria, though an established marker in the diagnosis of CKD, the use is limited because of a high false-positive rate due to variations.[32] Albumin is not a sensitive marker for CKDU compared to KIM-1 as albumin can detect only 62% of CKDU and only 32.4% has albumin:creatinine ratio (ACR) level above 300mg/g.[33] In the present study serum samples are taken as they are more stable than urine which may be affected by other influencing factors like urinary output, the timing of sampling collection and storage temperature.[34] The current study reveals that serum KIM-1 and NGAL have shown elevated levels in CKD and CKDU in comparison to control. In comparison to CKD, CKDU has shown significantly elevated levels of serum KIM-1 and NGAL. Previous work findings suggest serum NGAL correlated positively with renal disease-related clinical parameters predicting renal function decline,[34] as an ideal marker for AKI.[36] Studies also shown that urinary NGAL concentration highly correlated with serum creatinine level, GFR and proteinuria.[36] Blood KIM-1 and NGAL levels are independent risk factors for the progression of ESRD in CKD patients.[13] NGAL is also known as lipocalin-2 or siderocalin. It is a 25kDa glycoprotein synthesized in granules of neutrophils.[10,35] It is an important molecule triggering kidney development, converting embryonic mesenchymal cells into epithelial cells for forming tubules and complete nephrons.[8] It is present in renal proximal tubule cells, endothelial cells and smooth muscle and has recently been found to be expressed in patients with acute and chronic inflammatory diseases, ischemic diseases, metabolic diseases, and acute and chronic kidney failure.[10,35,36] NGAL mediates the mitogenic effect of epidermal growth factor receptor (EGFR) signaling.[8] Activation of EGFR associated with stimulation of hypoxia-inducible factor and expression of LCN2 results in renal damage and CKD progression.[8] It is a key mediator of tubular damage and progressive renal injury, with overexpression able to identify those at risk of rapid progression of CKD.[13] KIM-1(T-cell immunoglobulin, mucin containing molecule) is a Type1 transmembrane glycoprotein found in apical membrane of proximal tubule cells.[8] KIM-1 has been shown to be upregulated in differentiated proximal tubule epithelial cells in kidneys after ischemic or toxic injury.[8] Since KIM-1 ectodomain (91kDa) is cleaved from protein molecule by metalloproteinases in response to hypoxia, ischemia or toxic damage to renal tubules is manifested. However, it is not detected in healthy kidneys.[8] Upregulation of KIM-1 is a well-known consequence of proximal tubular damage in the nephron.[18] This study explains the limitations of using serum creatinine, serum urea and serum uric acid for screening CKDU while an advantage in investigating serum KIM-1 and NGAL levels. In CKDU patients, both serum KIM-1 and NGAL levels were found to be significantly higher with good sensitivity and specificity indicating possible early detection of CKDU. However, serum NGAL elevation was 4.8 fold in CKD and 6.7 fold in CKDU whereas serum KIM-1 elevation was 51.5 fold in CKD and 87.2 fold increase in CKDU. In a study of serum NGAL levels in pulmonary embolism, ROC analysis showed a cutoff value of 50 ng/mL with a sensitivity and specificity of 98.3% and 100% respectively.[31] KIM-1 showed AUC (0.74) and NGAL AUC (0.82). Though, the sensitivity and specificity are better in NGAL compared to KIM-1, due to less variation as shown in the histogram, KIM-1 can be a better marker for differentiating CKD with CKDU indicating better performance in diagnosis. Therefore, this study clearly emphasizes that serum KIM-1 is more sensitive and specific when compared with serum NGAL. There are few possible limitations of the present study. Serum creatinine was taken as the gold standard for screening, which may be influenced by nonrenal factors. The details other than diabetes and hypertension, exposure to various risk factors for CKDU, like nutrition, environmental factors and agricultural chemical details were not recorded.

5. CONCLUSION

The credibility of screening CKDU by means of serum creatinine, which is the current diagnostic method, has been

questioned due to its limited sensitivity and high probability of error. No previous studies have assessed the sensitivity and specificity of currently used screening markers for CKDU in Uddanam region, India. This study concludes that serum KIM-1 and serum NGAL are sensitive biomarkers for CKDU compared to conventional biomarkers and KIM-1 can differentiate CKD and CKDU.

6. AUTHOR'S CONTRIBUTION STATEMENT

Rajyalakshmi N conceptualized, designed the study and collected the data with regard to this work. Dr.Senthilkumar S and Dr.Vijaya S discussed the methodology and provided inputs towards designing of the manuscript. Dr. Vijayaraghavan R curated the data, prepared the original draft and analyzed the data. All authors read and approved the final version of the manuscript.

7. STATISTICAL ANALYSIS

The data obtained were analyzed using Sigmaplot 14.5 Version (Systat software Inc, USA). Data were presented as the mean ± standard error. One Way analysis of variance(ANOVA) with Bonferroni 't-test was used for the significance of the difference in the means between groups. The receiver operating characteristic (ROC) curves were used to determine the clinical accuracy of target markers. The area under curve (AUC) with 95% confidence interval was used for sensitivity and specificity. P-value < 0.05 were considered significant.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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