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Serum and urinary NGAL and Cystatin C levels as biomarker tools for diagnosis of both AKI and CKD: A histobiochemical comparative study

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Abstract: Introduction: Acute kidney injury (AKI) has a high global incidence with observable complications in critically ill patients. Long-term disease and medication complexity contribute to devastating chronic kidney disease (CKD) and diminish quality of life. Establishing new biomarkers would guide patient care and facilitate novel therapeutics development. **Methods:** Serum and urinary levels of creatinine, CysC, and NGAL were estimated in 86 renal patients and compared with healthy controls for AKI and CKD categorization. Creatinine and Cystatin C (CysC) measurements were used to estimate GFR. Biopsy tissues were prepared for light microscopy for further characterization. Patients' demographic data were used in group association study. **Results:** Thirty-six patients met the criteria for AKI and 50 for CKD. Mean values of serum CysC were higher than controls but similar in both disease states, while urine levels were slightly high in CKD patients and remained steady by the end of follow-up (EF-Up). Further, a 2.7-fold and 5.5-fold increase in serum NGAL were observed in AKI and CKD, respectively, and a dramatic 7.0-fold reduction in AKI group at EF-Up. Similarly, urine NGAL for AKI and CKD increased 3-fold and 6-fold respectively on admission, which multiplied to 7.3-fold and 10.7-fold at EF-Up. ROC assessment curve revealed a relatively higher NGAL performance at good predictive values than CysC ($p< 0.009$). **Conclusions:** We showed a higher urine NGAL and CysC sensitivity and specificity than their serum counterparts, providing a powerful discriminative tool between AKI and CKD. CysC, however, displayed less sensitive performance than NGAL, indicating effects by enigmatic non-specific factors.
Abbreviations: AKI (Acute kidney injury); CKD (Chronic kidney injury); CysC (Cystatin C); Neutrophil gelatinase associated lipocalin (NGAL).
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Keywords: Acute kidney injury (AKI), chronic kidney disease (CKD), Biomarkers, Cystatin C (CysC), NGAL

1. Introduction

Acute kidney injury (AKI), also termed 'acute renal failure' (ARF) is a condition characterized by rapid decline in renal function, accompanied by distinct kidney pathology and structural alternations. Several investigations have provided evidence that AKI presents with severe short- and long-term complications, principally, chronic kidney disease (CKD), pulmonary edema, cirrhosis, permanent kidney failure, and paralysis. [1] Additionally, there are associated lengthy hospital stays, high healthcare costs, and increased morbidity, with an independent risk factor of mortality. [2,3]

Despite several advances in the understanding of the pathogenesis and treatment of AKI, 5% of hospital admissions and 30% of ICU admissions result from a diagnosis of AKI. [3] Reports have indicated that hospital mortality for patients with AKI vary from 14-60%. [4,5] Epidemiological studies carried out recently demonstrated a wide variation in etiologies and risk factors associated with AKI and suggested a link to the consequent development of CKD and advancement to dialysis dependency. [6]

The development of CKD is becoming a major global public health challenge. The current burden of the disease might be due to a lack of understanding of the basis of its pathogenicity. Current evidence implies that high blood pressure (HBP) and type 2 diabetes (T2D) are worldwide, two significant illnesses linked to AKI and related kidney diseases. [7] Communities and many physicians, however, are not aware of the disorder. [8] In recent years, substantial efforts have been made to detect progressive kidney disease with selectivity and sensitivity. In 2002, the Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF)

developed a clinical guideline for the diagnosis of CKD. [9] According to this guideline, CKD is defined as either kidney damage or estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2 for three or more months with or without evidence of kidney damage, irrespective of the cause. [9]

However, elevated urinary levels of interleukins (IL-6, IL-8, IL-18), [10] neutrophil gelatinase-associated lipocalin (NGAL), [10] N-acetylglucosamine (NAG), and kidney injury molecule-1 (KIM-1) [10] provide a reliable evaluation of the intensity of AKI.

The discrepancy between the degrees of histopathological abnormalities and the extent of GFR decline was considered to be one of the central paradoxes in CKD and AKI, such that, the S3 segment of the proximal tubule, has become a critical region of evaluation as the dominant site of injury in human Acute tubular necrosis (ATN). Meanwhile, the relative contribution of damage to the adjacent distal tubule is unpredictable until there is a precise determination of the relative loss of medullary tissue in the CKD biopsies, including epithelial cells stripping into tubular lumen, brush border loss, and tubular casts formation.[11]

The classical methods of evaluation of renal function and various renal diseases implicate the measurement of blood urea nitrogen (BUN) and serum creatinine and the use of specific biomarkers. The problems encountered with these techniques are as follows: 1) BUN is dependent on non-renal factors, such as renal tubular handling, protein intake, catabolic state, volume status, gastrointestinal bleeding, unconnected with kidney function [12], and thus difficult to interpret outcome. 2) Creatinine-based criteria for AKI often disregard the fundamental critical serum fluid shifts or overload and renal reserve, which might present considerable variation in the GFR. 3) Urine creatinine measurement is an essential clinical marker, but it could also be associated with other conditions such as urinary tract obstruction and diabetes-associated complications, making the method least specific and misleading interpretation. 4) Substances such as bilirubin or certain drugs might alter the analytical outcome of creatinine quantitation, typically, with Jaffe-based tests. [13]

Further, biomarkers such as alanine aminopeptidase (AAP), localized in the proximal tubular brush border villi, Angiopoietin-1/2, secreted by endothelial cells, which is upregulated in glomerular disease, but also, in malignancy, and in inflammatory diseases.[14] Despite enabling the detection of subtle alterations in renal function before serum creatinine became noticeable, their effects were only pronounced in patients with other complications, reflecting disease severity, rather than assay sensitivity, making these methods unreliable.

Currently, biomarkers for the detection of kidney diseases such as neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) have been discovered in preclinical studies and shown to be produced predominantly by the injured kidney, which appears in the urine at high levels before creatinine detection. [15,16]

NGAL is a 25-kD protein belonging to the lipocalin family. Its rising levels in AKI has been well-documented in the serum and urine of human ischemic and nephrotoxic injuries, and animal disease models, hence, allowing the determination of the protein as a novel biomarker in these body fluids.[16]

Similarly, urinary excretion of CysC reflects explicitly tubular damage, as the systematic production of the protein in urine is atypical. However, recent reports have provided insight into the augmentation of urinary excretion of CysC in albuminuria (excessive protein leakage into urine), which could occur in other disease states such as T2D and cardiovascular diseases (CVDs). [17] Thus its evaluation in AKI might require confirmation with other determinations, such as NGAL levels and renal biopsy examination.

Our present studies sought to: 1) Predict how renal patients' demographics and clinical data associate with renal disease. 2) Test whether serum and/or urine levels of NGAL and CysC are able to detect the early phase of AKI from CKD, compared with the routine creatinine levels. 3) Employ the creatinine and CysC data to determine eGFR. 4) Evaluate histopathological changes of the renal tissue biopsies and correlate outcome with the serum and urine assays to gain better insight into the pathogenesis of the renal diseases. 5) Demonstrate correlations between biomarker levels and clinical outcomes, such as the need for dialysis and the histopathological findings.

2. Material and Methods

2.1. Study Design

We carried out a hospital-based, single-center biochemical and histopathological descriptive study, conducted in Al Noor General Specialist Hospital, Makkah, Kingdom of Saudi Arabia (KSA). Participants were patients admitted to the outpatient internal medicine and nephrology departments at Al Nour Specialist Hospital with a total of 86 patients, mean age 51.4±11.6 years (range=36-69), comprising 60 (70%) males and 26 (30%) females. Informed consent was obtained from each participant prior to data and samples collection according to Helsinki declaration.

2.2-The inclusion/exclusion criteria and controls

Information obtained from patients' medical records was used to determine eligibility, as follows: 1) Existing diagnosis of renal disease. 2) Kidney function of each patient was assessed routinely by serum creatinine, baseline >3.0 mg/dL, according to the Helsinki Declaration of the World Medical Association (WMA). [18] 3) The possibility of categorizing patients into CKD and AKI, based primarily on the extent of kidney disease, measured by serum creatinine and CysC on admission and at the end of the 12 months follow-up period. Patients with end-stage renal disease (ESRD), overlapping syndromes, malignancies, pregnancy, and hemodialysis or kidney transplantation in their medical records were excluded. A control group comprising 30 healthy volunteers from the hospital medical staff participated in the study.

2.3-CKD and AKI categorization Criteria

The Kidney Disease: Improving Global Outcomes (KDIGO, 2017) guidelines for clinical practice and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) criteria [9] were followed for the classification of CKD and AKI. We utilized 24-h creatinine clearance as an indicator of GFR. CKD was defined by a decline in patient's eGFR <90 ml/min/1.73 m^2 as the reference range and an outpatient baseline. Serum and urine creatinine were assayed by a modified Jaffe reaction. [19] We used the CKD-EPI-creatine and CysC mix equation (CKD-EPI-creatinine-CysC)[9] to estimate GFR (eGFR; ml/min/1.73 m^2). Serum and urine CysC and NGAL levels were determined for each patient and healthy participants and mean controls for each determination were used as a reference for comparisons with corresponding patient values.

2.4-Study approval

The protocol for this study was approved by the Biomedical Ethics Committees College of Medicine in Umm Al Qura University, and the Saudi Arabia Ministry of Health, before the research began.

2.5-Patient demographic and clinical data collection

Patient prepared questionnaires regarding demographics, such as age, sex, occupation, and socioeconomic status, as well as dialysis information, baseline creatinine, and body mass index (BMI) measurements were obtained from medical records. The BMI each patient was used to determine underweight and obesity.

2.6-Serum and urine sample preparation and biomarker detection

Blood and urine samples (4 ml and 10 ml, respectively) were collected from each patient on admission and at the EF-Up. Samples were prepared for creatinine by the modified kinetic Jaffe reaction (Siemens Healthcare Diagnostics Inc., Newark, DE) with a limit of detection 9 µmol/L. For measurement of serum and urine CysC and NGAL, we used the Elabscience ELISA kits (Elabscience Ltd, Wuhan, China; CysC ELISA kit [Cat# E-EL-H0055]; NGAL ELISA kit [Cat# E-EL-H0096]), following the manufacturers' protocol. We performed our analyses in a blinded fashion. The inter- and intra-assay coefficient variabilities (CVs) were 5.6% and 6.5% respectively, which corresponded to the kit manufacturer's report.

2.7-Renal biopsy preparation for histopathological studies

We obtained each of the patient renal biopsies of 1 cm length and 1.2 mm diameter, with an average 10 glomeruli per biopsy, using 16-gauge needles under real-time, ultrasound-(US) guidance, without bleeding complications. Biopsies were processed for H&E and PAS staining and examined under a light microscope to enable us to diagnose focal glomerular disease precisely.[20]

2.8-Statistical analysis:

The collected laboratory data were coded, tabulated, and statistically analyzed using IBM Statistical Package for Social Sciences (SPSS) software version 22.0 (IBM Corp., Chicago, USA, 2013). Descriptive statistics were performed for quantitative data as minimum & maximum of the range, as well as mean ± SD (standard deviation) for quantitative parametric data, while qualitative data were estimated as numbers and percentages. Demographic and recorded patient clinical data were compared between patients who developed AKI and/or CKD using either unpaired t-test or the Fisher's exact test. Two-tailed p-values are presented. Mean and standard deviation estimations were performed to measure both serum and urine creatinine, NGAL and, CysC. Mean outpatient admission (OP-A) values were compared with mean values obtained at the end of follow up period (EF-Up). At both OP-A and EF-Up timepoints, mean creatinine, NGAL and CysC values among AKI and CKD patients were compared using two-tailed unpaired t-tests. Fisher's exact test with two-tailed p-values was used to compare differences between groups. A p-value of ≤ 0.05 was considered to be statistically significant. Receiver operating characteristic (ROC) curve data were obtained for both patient serum and urine NGAL and CysC determinations to assess assay sensitivity, specificity, and accuracy.

3. Results

All of the 86 patients presented with evidence of renal disease were enrolled in the study in 2017 for a 12 months period. These patients displayed a preexisting decrease in GFR <90 ml/min. Proteinuria was present at baseline in 22.1% (n=19) of patients

and did not differ between the classified groups (data not shown). Male presentation dominated the patients (n=60), accounting for AKI (n=15) and CKD (n=45). Study demographic and clinical data obtained from patient records are shown in Table 1. There was no significant difference between the AKI and CDK groups regarding age (mean patient ages: AKI, 51.4±11.6 and CKD, 51.1±9.7). Creatine measurements were repeated and compared with two new biomarkers (CysC and NGAL). Creatine and CysC data were employed in the CKD-EPI-creatinine-CysC mix equation to estimate patient GFR (eGFR). Dialysis was required by 57 patients and was used more frequently in CKD (n=50) than AKI (n=7), Table 1.

BMI of <18.5 kg/m^2 was considered underweight, 18.5-24.9, normal, 25-29.9, overweight, and ≥30, obese. Fisher's exact test was employed to study the relationship between body mass index (BMI) and AKI or CKD. BMI was used as an independent variable; adjusted variables included age, gender, and socioeconomic status, and baseline eGFR. Analyses of interactions between different age groups and BMI using continuous or categorized variables showed non-significant relationship in the prevalence of AKI and CKD (p=0.214), Table 1.

Examination of the relative risk for the prevalence of AKI and CKD between different occupations and 'no work' also revealed a nonsignificant association with any type of work activity (heavy, light, very light). However, there was a dramatic 3-4-fold reduction in the prevalence of AKI with 'no work,' but not with CKD (Table 1).

At 95% CI, the mean serum creatinine level did not increase in AKI patients and was increased by only 2-fold in CKD (p=0.001), compared with controls. Similarly, urine creatinine levels increase by less than 2-fold in both AKI and CKD patients (p=0.016). At the EF-Up, there was a dramatic 3.8-fold increase in the mean urinary creatinine levels of the CKD patients, compared to controls. However, no considerable change in the serum levels for both disease states (p=0.001) and urine AKI (p=0.001) was observed. Thus, the difference between the AKI and CKD urine creatinine determination at EF-Up makes the urinary determination relevant in discriminating tubulopathies.

Though CysC has been shown to be a better indicator of changes in GFR than serum creatinine, [21] the available literature reference values vary, ranging from serum: 0.51-2.14 mg/L [21,22] and urine: 0.03-0.723 mg/L determinations. [23,24] Our Saudi Arabian control mean serum CysC level at 95% CI was within previous determinations (0.8±0.1 mg/dL; p=0.001). Compared with our patients, there was a significant 7.3-fold CysC increase in AKI and 8.2-fold increase in CKD. Further, urinary CysC data revealed an efflux of the biomarker in patient urine samples, with a dramatic elevation of 11.9-fold increase in AKI and 13.2-fold in CKD (p=0.06), compared with controls. These trends of both serum and urinary CysC levels did not change appreciably with controls and disease states at EF-Up.

Serum NGAL reference values in the literature range from 100-117.5 ng/ml. The concentration surpassing this level indicated AKI or CKD. [16,25] Urinary reference ranges from 2-5 ng/ml, above which kidney dysfunction is designated.

Our mean control serum NGAL concentration was 36.65±4.21 ng/ml and urine NGAL of 2.01±0.42 ng/ml (Table 2). Serum NGAL control values were lower than quoted literature values, which we utilized as a reference to make a comparison with patients' values to support our classification of AKI and CKD, in addition to our eGFR and histopathological findings. We observed a 2.87-fold increase in the serum of AKI and 5.53-fold increase in CKD patients on admission, compared with controls (p=0.001). This trend also did not change appreciably with CKD patients at EF-Up but was reduced dramatically with AKI patients (p=0.001). Similarly, there was a 3.18-fold increase of urinary NGAL in AKI patients and 6.12-fold increase in CKD patients on admission, compared with controls (p=0.001). This trend in urinary NGAL multiplied, yielding 7.34-fold increase in AKI patients and 10.72-fold increase in CKD by EF-Up (p=0.001). These renal dysfunction outcomes observed with NGAL levels in both AKI and CDK patients correlated with the diminished eGFR (p=0.001) on admission, which did not change appreciably at EF-Up.

Unfortunately, our eGFR data did not reveal dramatic differences between AKI and CKD patients both on admission and EF-Up, although, compared with the controls, there were vast differences with the two disease states, signifying the need to utilize supporting parameters in renal disease classification. Our NGAL data, favorably, demonstrated marked differences, unveiling the severity of CKD, compared with AKI, which, positively correlated with more CKD patients greater need for kidney dialysis, (AKI: n=7; CKD: n=50), Table 1.

ROC curve analysis for serum and urine NGAL and CysC levels performed for relative to discrimination of sensitivity and specificity of their detection (Figure 1; Table 3). NGAL measurements demonstrated overall higher performance in both serum and urine, at a better AUC range than CysC, with serum NGAL sensitivity being 20% > CysC and urine, 35% > CysC at 95% CI and higher predictive values at a cut-off point of log 3.5 units (Figure 1). This enabled a precise assessment of the progression of kidney

impairment to support patients' categorization into AKI and CKD.

Table 1: Comparison between the two studied groups regarding demographic data.

	Group I (AKI) n=36		Group II (CKD) n=50		Control n=30		P
Age (years)							
Range	34-69		36-68		32-65		0.621
Mean ± SD	51.4±11.6		51.1±9.7		50.3±8.22		
Sex	n	%	n	%	n	%	
Male	25	69.4	35	70.0	16	53.3	0.478
Female	11	30.6	15	30.0	14	46.7	
BMI category (kg/m^2)							
Underweight (UW)	10	27.8	12	24.0	6	20.0	
Normal weight	5	13.9	11	22.0	8	26.7	0.214
Overweight (OW)	9	25.0	16	30.0	10	33.3	
Obesity (OB)	12	33.3	11	22.0	6	20.0	
Residence							
Rural	17	47.2	27	54.0	17	56.7	0.336
Urban	19	52.8	23	46.0	13	43.3	
Occupation							
Light worker	13	36.1	14	28.0	7	23.3	
Hard worker	10	27.8	11	20.0	8	26.7	0.085
House wife	10	27.8	13	26.0	9	30.0	
Not working	3	8.3	12	20.0	6	20.0	
Marital status							
Married	21	58.3	37	74.0	24	80.0	
Single	7	19.4	7	14.0	3	10.0	0.072
Others	8	22.2	6	12.0	3	10.0	
Socioeconomic status							
High	13	36.1	16	32.0	10	33.3	
Moderate	9	25.0	16	32.0	8	26.7	0.226
Low	14	38.9	18	36.0	12	40.0	
# of patients needed Dialysis	7	19.4%	50	100%	0	0%	0.000

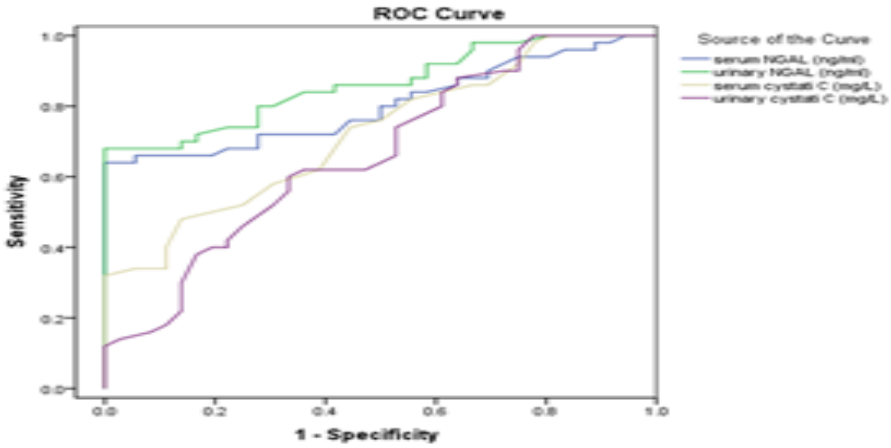

Figure 1. Receiver operator characteristic (ROC) curve for serum and urine NGAL and CysC of renal disease patients. The determined area under the curve (AUC) for serum NGAL was 0.804 (95% confidence interval: 0.712–0.896), urine NGAL was 0.862 (95% confidence interval: 0.787–0.937), serum CysC was 0.724 (95% confidence interval: 0.619–0.829), urine CysC was 0.666 (95% confidence interval: 0.549–0.782). The biomarker log cut-off was 3.51 units.

Table 2: Comparison of association between the AKI and CKD studied groups with lifestyle habits

	Group I (AKI) n=36		Group II (CKD) n=50		Control n=30		P
Smoking Status	n	%	n	%	n	%	0.208
Smoking	15	41.7	21	42.0	12	40.0	
Ex-smoker	4	11.1	10	20.0	10	33.3	
No	17	47.2	19	38.0	8	26.7	
Salt containing food							0.145
Yes	22	61.1	32	64.0	20	66.7	
No	14	38.9	18	36.0	10	33.3	
Canned foods							0.038*
Yes	32	88.9	37	74.0	25	83.3	
No	4	11.1	13	26.0	5	16.7	
Fast food							0.370
Yes	25	69.4	33	66.0	15	50.0	
No	11	30.6	17	34.0	15	50.0	
Source of drinking water							0.411
Tap water	15	41.7	21	42.0	17	56.7	
Bottle water	21	58.3	29	58.0	13	43.3	
Disease duration "months"							0.301
Range	10-82		15-83		-		
Mean ± SD	50.1±20.4		47.9± 19.1				

Table 3: Comparison between the studied groups regarding serum and urine NGAL, CysC and Creatinine levels with subsequent eGFR values.

	Group I (AKI) n=36	Group II (CKD) n=50	Control group n=30	P
NGAL *On Admission*				
Serum (ng/mL)	105.1±32.1	203.0±90.2	36.65±4.211	0.001*
Urinary (ng/ml)	6.4±2.3	12.3±4.3	2.01±0.42	0.001*
At the end of follow-up				
Serum (ng/mL)	14.8±2.3	211.4±97.5	37.01±3.33	0.001*
Urinary (ng/mL)	15.2±4.0	22.2±5.8	2.07±0.22	0.001*
CysC *On Admission*				
Serum (mg/L)	0.8±0.1	0.9±0.1	0.11±0.031	0.001*
Urinary (mg/L)	4.1±1.2	4.9±1.2	0.34±0.014	0.001*
At the end of follow-up				
Serum (mg/L)	0.8±0.1	0.9±0.1	0.13±0.07	0.001*
Urinary (mg/L)	4.4±1.4	4.9±1.4	0.37±0.05	0.061
Creatinine *On Admission*				
Serum (mg/dL)	0.8±0.3	1.5±0.7	0.62±0.22	0.001*
Urinary (mg/dL)	1.2±0.5	1.2±0.4	0.71±0.28	0.016*
At the end of follow-up				
Serum (mg/dL)	0.7±0.3	1.7±0.8	0.63±0.15	0.001*
Urinary (g/day)	1.1±0.4	3.1±0.9	0.73±0.13	0.001*
eGFR (mL/min/1.73m^2)				
On Admission	58.7±4.26	48.6±6.98	91.6±4.02	0.001*
At the end of follow-up	55.05±10.11	44.73±4.15	94.8±8.72	0.001*

Table 4: ROC curve data of both serum and urinary NGAL and CysC levels.

Test Result Variable(s)	Area	P value	Sensitivity (%)	Specificity (%)	Accuracy (%)	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
Serum NGAL (ng/mL)	0.804	.0001*	84.0	79.0	82.0	.712	.896
Urinary NGAL (ng/mL)	0.862	.0001*	88.0	80.0	82.3	.787	.937
Serum cystatin C (mg/L)	0.724	.000	70.0	80.0	74.0	.619	.829
Urinary cystatin C (mg/L)	0.666	.009	65.0	70.0	68.0	.549	.782

[ns] not significantly different compared to reference group (p>0.05).

Glomerular disease could exist with near-normal creatinine (2.2 mg/dL), proteinuria (4.8 g/day),[17] and even near normal levels of other more sensitive biomarkers. Hence, we found it necessary to utilize patient kidney biopsies as a means of further disease classification. Due to ethical considerations, biopsies became available only in suspicious cases of CKD or unknown renal disease and occasionally from donor kidney biopsies (Figure 2).

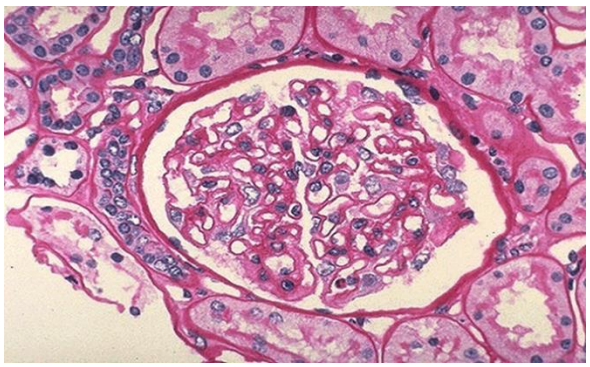

Figure 2. Periodic Acid Schiff (PAS) stain of a donor renal biopsy sections, observed by light microscopy. Section highlighting the thin basement membrane of well-defined glomerular capillary loops of endothelial cells and the tubular epithelium, mesangial area is normal in size. Podocytes are present, forming visceral epithelial surface. Bowman's space is apparent along with parietal epithelial cells. (x400).

In view of these limitations, most of our examined tissues, using H&E and PAS staining, revealed histopathological features of CKD, as displayed as representative tissue sections in Figure 4. However, two of our unknown disease biopsies were later diagnosed as AKI (Figure 3).

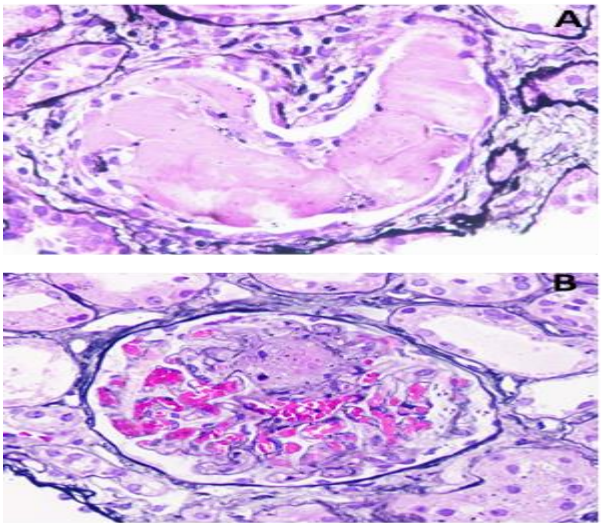

Figure 3.A) Periodic Acid Schiff (PAS) stains of kidney biopsy of a 50-year-old male, diagnosed as acute kidney injury (AKI) patients, observed by light microscopy. The section shows diffuse acute tubular injury, characterized by marked attenuation of the tubular epithelium with missing of the proximal tubular brush borders also here is evidence of mesangial cell proliferation, mesangial cells proliferation is apparent, with excessive mesangial matrix production, compressing the glomerular capillary lumina, excessive matrix production and tubular lumens show typical globular, retractile, brightly eosinophilic and fuchsinophilic casts, (x400).

Figure 3.B) PAS stain of renal biopsy section of a male patient; aged 35 years with AKI showing

enlarged glomeruli, displaying visceral epithelial cells, abundant vacuolation, and foam-forming cytoplasm. glomeruli are enlargement, visceral epithelial vacuolation, and foamy cytoplasm (x400).

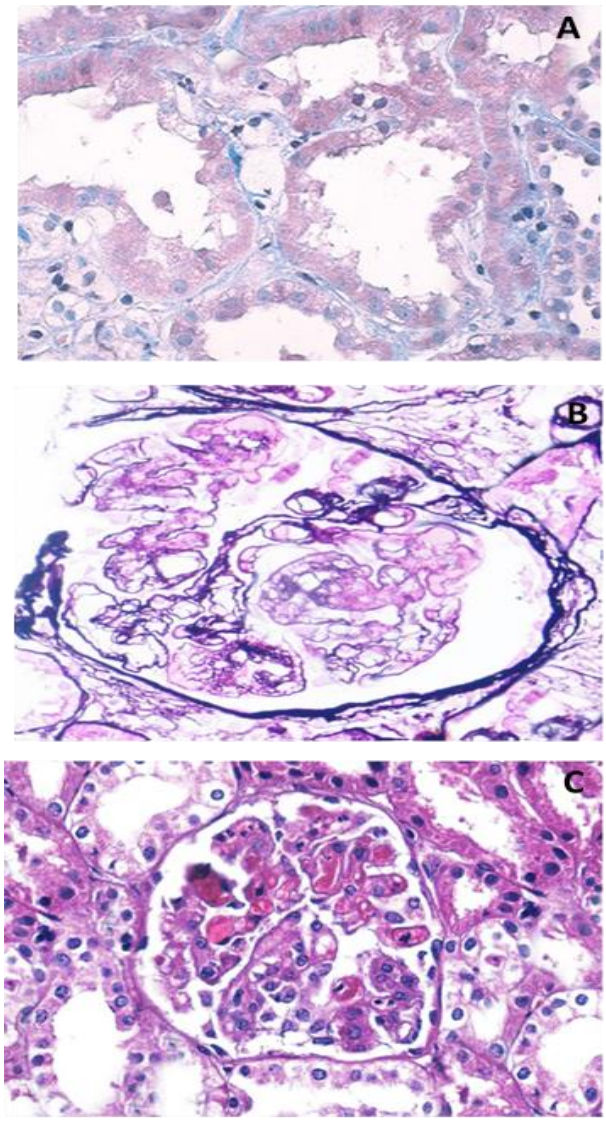

Figure 4A is an H&E stain of renal biopsy of a 56 years old male with hepatorenal syndrome type CKD. There is acute tubular necrosis, characterized by thinning of the proximal tubular epithelial cells and widening of the tubular lumens with no evidence of glomerular abnormalities (x400). **Figure 4B** is a PAS stain of kidney biopsy from CKD of a 44 years old male, showing foreign-body emboli in interlobular arteries, characteristic of crystal embolism (CCE) able of causing a sharp or chronic decline in renal function due to sporadic presence of erythrocytes in the lumina, arcuate vessels, interlobular arteries, and the glomeruli. **(x400). Figure 4C** is a PAS stain of percutaneous kidney biopsy from male patients with CKD aged 44 years, which reveals intra-arteriolar foreign-body emboli with an associated ischemic collapse of the glomerulus and tubular epithelial vacuolization. **(x400).**

4. Discussions

Acute kidney injury (AKI) is caused by a spontaneous event such as ischaemia, nephrotoxins harmful bacteria infection (sepsis) and a life-threatening complication of septic shock, liver transplantation, extreme dehydration, blood loss resulting from major surgery, and is typically reversible. [3] CKD, by contrast, usually results from a long-term condition, like T2D, HBP, renal carcinomas, as well as medications with potential of causing slow kidney damage and its function to decline.[4,5] Thus to treat kidney failure effectively, it is critical to unveil whether the kidney disease developed instantaneously (acute) or persistently (chronic). CKD is an emerging worldwide public health challenge and demands multilevel early support, as the disease is not diagnosed early enough to initiate treatment regimens in order to minimize mortality and morbidity rates. Additionally, most people are unaware that they have counteracted the disease due to late detection and intervention. Accordingly, a biomarker of kidney damage with high sensitivity and selectivity would be useful for screening and identifying patients with increased risk of disease, diagnosis, progression, therapy, and effective management to curtail the incidence of end-stage renal disease (ESRD) and lift the burden, as well as costs incurred in CKD.

Our present study found no significant difference between AKI and CKD patients regarding our demographic data, (age, occupation, and socioeconomic status).

Rubinstein and coworkers [26] examined the relative risk for the occurrence of CKD between different occupations in the US adult population, showing pressure-related occupations such as healthcare support, legal practitioners, manufacturing, and food productions association with risk in developing CKD than least-pressured or light work, and attributed the basis of this outcome with stress. Contrary to their results, we found no associations between any form of work (hard or light) with the prevalence of both AKI and CKD, possibly due to the small size of our study participants. We, however, observed a 3-4-fold negative correlation between individuals without jobs with the prevalence of AKI.

Low socioeconomic status (SES) has been correlated to both the development and progression of chronic CKD. The impact of SES on the severity of CKD at presentation to a renal service is unclear. Bello and coworkers [27] investigated the relationship between

SES and severity of CKD in a retrospective, cross-sectional analysis involving numerous patients and found that low SES is related to the severity of CKD at presentation. Further, Garcia-Garcia and Jha [28] reviewed the principal links between poverty and CKD and the consequent implications for the prevention of kidney disease, as well as proper maintenance of individuals living in disadvantaged communities with the burden of unrecognized and untreated CKD. Their study found that more than 70% of patients with CDK live in low-income communities. [28] Surprisingly, our study found no significant association between varying socioeconomic status and AKI or CKD prevalence.

Likewise, obesity did not significantly associate with a higher prevalence of AKI and CKD in our evaluation. Overweight and obesity have always been linked with T2D, which, in turn, has a close association with CKD and a critical risk factor of ESRD.[29,30] Nevertheless, current reports have indicated that there are distinct etiologies for both underweight and overweight/obesity relatedness to CKD: While diabetes mellitus (DM) and hypertension have been observed among overweight/obese CKD patients,[31] particularly in males, cancer prevalence has been noted among underweight CKD patients.[32] Unfortunately, we were unable to perform extensive comorbidity association studies with our patients to enable validation of these previous reports.

A thorough view of the literature suggests a lack of standardization in defining both AKI and CDK, possibly due to the diversified comorbid conditions associated with renal disease in general. We performed measurements on new markers and compared their sensitivity and specificity in the prediction of AKI and CKD. We utilized the CKD-EPI-creatinine-CysC mix equation to measure eGFR as part of our disease classification. [33] CysC is a protein encoded by the CST3 gene and produce by all karyocytes in the body and excreted at a constant rate from the blood by the kidneys as it passed through glomerular filtration and metabolized after tubular reabsorption in proximal convoluted tubule,[34] making it a good candidate for screening and monitoring kidney dysfunction.

Reference values of serum CysC range from 0.5-0.98 mg/L and from for freshly collected urine samples, from 0.03-0.18 mg/L. The values of our urine determinations were a bit higher than the previous reports, which could have an ethnicity connotation (Table 2 and Table 3). Yet the levels were inconceivably high in the AKI and CKD patients, compared with the controls and consistent with renal tubular dysfunction, [21] making CysC beneficial for a rapid screen of kidney functionality. As our mean serum creatine levels were significantly higher for the CKD patients, we were able to use both of our creatinine and CysC data to demonstrate a mean eGFR< 60 ml/min/1.73m^2 in both AKI and CKD with enhanced sensitivity, after adjusting for body surface.

NGAL has proven to be a useful biomarker in CKD with an ideal potential for early detection of the disease.[35] The protein is produced systemically in response to kidney damage as a result of injury to renal tubules, and hence, its expression levels indicate the extent of kidney damage.[36] Our study showed a positive correlation of NGAL levels with eGFR determination and the ability to detect this protein in patients with modest renal impairment, consistent with other reports.[35,37] Our ROC determinations also revealed high sensitivity and selectivity of NGAL measurement, and therefore, a base-tool to predict AKI and CKD during OP-A by providing prognostic clues of tubular damage.

Gathering available information on a renal biopsy to enhance patients' benefit, as well as the epidemiologist and researcher is a challenge that faces the nephrology community. Characterizing AKI and CKD, particularly in stages, is an essential part of rational management, and renal biopsy plays a crucial role in determining the underlying processes. There are no available global guidelines for the renal researchers on indications, diagnostic, prognostic, and this relatively safe test.[14] Obtaining renal biopsy and the subsequent determination of light and transmission electron microscopy (TEM), as well as immunofluorescence and microarray techniques, is a powerful tool for predicting CKD progression and response to therapy. Although we did not find much success with our TEM preparations or genetic testing due to limited biopsy sizes, our histopathology studies, using PAS and H&E staining revealed several glomerular spaces, glomerular basement membrane (GBM), and glomerular epithelial cells abnormalities, which were insightful for our disease classification.

Our study has several notable strengths. We enrolled 86 patients at our Renal Unit, suspicious of renal disease, with baseline creatinine levels for at least a year before inclusion in the study. We applied the sensitive AKIN and KDIGO-CKD criteria to discriminate between AKI and CKD patients. We studied demographic associations with each disease category and broadened our scope in disease determination and classification by employing new biomarkers and pathophysiology of kidney biopsies. We were limited, being unable to perform extensive studies on comorbidity associations, such as the link between diabetic nephropathy and HBP with AKI and CKD. Nevertheless, we determined a plausible link with weight abnormalities through BMI, using patient

BMI. Also, TEM studies of ultrastructural changes with patient biopsies would have been beneficial in the further determination of disease severity.

Our study has several notable strengths. As it is depend in several studies on experimental animals with different models of renal injury.[38,41]

Conclusion:

The utilization of eGFR, biomarker, and histopathology data are necessary for providing further confirmation of assigning the type and stage of kidney disease. Our examination of the role of NGAL with high sensitivity and specificity helped in the precise measurement of renal function and served, in part, as a tool capable of distinguishing AKI and CKD for prognosis purpose. Urine NGAL levels exhibited higher performance and predictive power (sensitivity and specificity) than serum NGAL, which discriminated between AKI and CKD and thus, a critical index based-tool. The order of performance, however, was that CysC was not as high as NGAL, indicative of CysC measurement being affected by other non-specific confounding factors.

Further, using renal biopsies besides reliable biomarkers to evaluate CKD cases or undefined renal disease exerts invaluable aspect of the overall diagnosis process, providing prognostic insight into specific morphological and molecular patterns of the disease for targeted therapy and for elucidating the mechanisms and pathogenesis of progressive renal injury.

Disclosure and Conflicts Statement

The author declares no conflicts of interest.

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