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Diagnostic accuracy of neutrophil gelatinase-associated lipocalin as a predictor of chronic kidney disease

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Abstract

Introduction

Chronic kidney disease (CKD) is a major threat to public health problem, in terms of prevalence of disease, cost of treatment, and the comorbidities involved. To screen out the severe disease earlier and more accurately, estimated glomerular filtration rate (eGFR) and albuminuria are still regarded as the ideal markers of kidney function. Neutrophil gelatinase-associated lipocalin (NGAL) is a new biomarker for detecting early kidney damage. The aim of the study was to assess diagnostic significance of NGAL in early stages of CKD among patients.

Patients and methods

We measured urinary neutrophil gelatinase-associated lipocalin (uNGAL) and serum neutrophil gelatinase-associated lipocalin (sNGAL) levels in 40 patients with stages 2 and 3 CKD and 20 controls by ELISA.

Results

There was a highly significant increase in uNGAL and sNGAL, albumin/creatinine ratio, and eGFR on comparison of all patients and groups 1 and 2 with the control group ($P < 0.001$) and on comparing group 2 with group 1. We found that uNGAL and sNGAL were highly significantly and positively correlated in the patient groups ($P < 0.001$, r = 0.924) and in group 1 and group 2 ($P < 0.001$, $r = 0.886$ and 0.875, respectively). We also found that uNGAL and sNGAL were highly significantly correlated with albumin/creatinine ratio and C-reactive protein in the patient group and in both group 1 and group 2 ($P < 0.001$), and they were significantly negatively correlated with eGFR in groups 1 and 2 ($P = 0.02$ and $P < 0.001$, respectively). The area under curve for uNGAL to identify patients with CKD and those with stage 3 CKD was 0.98 and 0.909, with sensitivity of 92.5 and 85%, respectively, and specificity of 90 and 82.5%, respectively ($P < 0.001$). The area under curve for sNGAL was 0.937 and 0.876, with sensitivity of 85 and 95%, respectively, and specificity of 85 and 80%, respectively ($P < 0.001$) and for C-reactive protein was 0.774 and 0.954, with sensitivity of 72.5 and 95%, respectively, and specificity of 60 and 80%, respectively ($P = 0.001$ and $P < 0.001$, respectively).

Keywords: Biomarker, chronic kidney disease, neutrophil gelatinase-associated lipocalin

Introduction

Chronic diseases are the most famous reason of death all the world, responsible for 60% of all causes of death, of which 80% deaths happen in low-income and middle-income groups [1]. Chronic kidney disease (CKD) is becoming a major and serious public health problem, leading to not only kidney damage but also multiple systemic disease. The prevalence of all stages in adults varies worldwide from 7 to 12% [2].

Early stages of CKD is defined as kidney damage or glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2 for at least 3 months. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies [3]. Early detection may help to slow the progression of kidney disease and consequently

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avoid kidney failure. Most people with CKD die owing to cardiovascular disease, as coronary artery disease causes 40–50% of deaths [4].

GFR is regarded as the optimal marker of kidney function. However, GFR evaluation is time consuming and is usually estimated from equations including serum creatinine and cystatin C [5]. Moreover, albuminuria [6] antecedes kidney function deterioration and has powerful relationship with disease progression and outcome.

However, it has low sensitivity, as it reflects a previously obvious destruction of the glomerular barrier. The rate of deterioration in renal function, in some CKD-associated diseases such as diabetic nephropathy, is linked with the degree of renal tubulointerstitial impairment more than with the severity of glomerular lesions [7,8].

Recently, neutrophil gelatinase-associated lipocalin (NGAL) is known as a new biomarker for detecting early kidney destruction [9]. Human NGAL protein was initially isolated from secondary granules of human neutrophils. It is a 25-kDa protein covalently bound to neutrophil gelatinase [10]. It is secreted by different systems such as gastrointestinal, respiratory tract, and kidneys. In spite of that plasma NGAL is directly filtered by the glomerulus, it is largely reabsorbed in the proximal tubules. It is secreted into the urine by the thick ascending limb of loop of Henle and collecting tubes of the kidney, with synthesis in the distal tubules [11].

The aims of the present study were to evaluate the expression of serum neutrophil gelatinase-associated lipocalin (sNGAL) and urinary neutrophil gelatinase-associated lipocalin (uNGAL) in early stages of CKD among patients and to assess its diagnostic significance as a biomarker of kidney.

Patients and Methods

This single-center study was performed in the National Institute of Urology and Nephrology, between August 2019 and March 2020. The study was approved by the local Bioethics Committee for Human Research. Before enrollment, and written informed consent was obtained from all participants. A total of 40 adult patients with CKD and 20 apparently healthy participants were enrolled in this study. All of them willingly participated in the study and were comparable regarding age and sex.

The patients were subclassified into two groups according to calculated estimated glomerular filtration rate (eGFR), using the abbreviated modification of diet in renal disease (MDRD) equation: GFR (ml/min/1.73 m^2)= $186\times(\text{creatinine}/88.4)^{-1.154}\times(\text{age})^{-0.203}\times(0.742$ if female). CKD stage 2 represented 60 less than GFR less than 89 and CKD stage 3 represented 30 less than GFR less than 59. Group 1 (stage 2) included 20 patients (12 males and eight females). Their ages ranged between 23 and 62 years (mean±SD: 38.85±12.41), with eGFR of 60–87 ml/min/1.73 m^2. Group 2 (stage 3) included 20 patients (11 males and nine females). Their ages ranged between 22 and 65 years (mean±SD: 40.65±13.13), with eGFR: 30–58 ml/min/1.73m^2.

All individuals were subjected to the following: full history taking, kidney function tests including serum creatinine, urea, C-reactive protein (CRP), and urine albumin/creatinine (Alb/Cr) ratio on Vitros 350 autoanalyser (Ortho Clinical Diagnostics, Mumbai, Maharashtra, India) and GP100 (Greiner Diagnostic GmbH, Bahlingen, Germany) according to routine standard methods. NGAL assays were carried out by a sandwich ELISA technique using reagents provided by FineTest (Wuhan Fine Biotech Co. Ltd, Hubei, China) according to manufacturer's instructions.

Intra-assay CV was less than 8%, and interassay CV was less than 10%. uNGAL and sNGAL assay was expressed as ng/ml.

Patients on dialysis, who underwent transplantation, or with histories of malignant disease, myocardial infarction, acute or chronic infectious disease, and chronic liver disease were excluded from the study.

Statistical analysis

Quantitative data were expressed as median and interquartile range, and analyzed by nonparametric tests (Mann and Whitney). We used Spearman's correlation. Receiver operator curve (ROC) analysis had been done to calculate the area under curve (AUC) and identify the optimal cutoff values, sensitivity, and specificity. Analysis was performed by statistical package software IBM SPSS, version 24 (IBM Corp, Armonk, New York, USA). All tests were bilateral, and a P value less than 0.05 was considered statistically significant.

Results

A comparison between patient and control groups is performed (Table 1). There was a highly significant increase in uNGAL and sNGAL ($P < 0.001$, Figure 1), Alb/Cr ratio, eGFR ($P < 0.001$), and CRP ($P = 0.001$).

On comparison of groups 1 and 2 with the control group, Table 2 shows a highly significant increase in uNGAL and sNGAL ($P < 0.001$, Figure 2) and Alb/Cr ratio, with P value less than 0.001. There are highly significant increases in uNGAL, sNGAL [Figure 2], Alb/Cr ratio, and CRP ($P = 0.001$, $P = 0.006$, $P < 0.001$, and $P < 0.001$, respectively) on comparing group 2 with group 1.

On comparing uNGAL and sNGAL, we found a highly significant and positive correlation in all patient groups ($P < 0.001$, $r = 0.924$) and in groups 1 and 2 ($P < 0.001$, $r = 0.875$ and 0.886, respectively) (Table 3 and Figures 3 and 4).

We also found that uNGAL and sNGAL were highly significantly correlated with eGFR, Alb/Cr ratio, and CRP in the patient group ($P < 0.001$) (Table 4) and in both groups 1 and 2 (Table 5). We found that uNGAL and sNGAL were highly significantly correlated with Alb/Cr ratio and CRP ($P < 0.001$, Figures 5 and 6). They were highly significantly negatively correlated with eGFR in group 2 ($P < 0.001$ and $P = 0.002$, respectively),

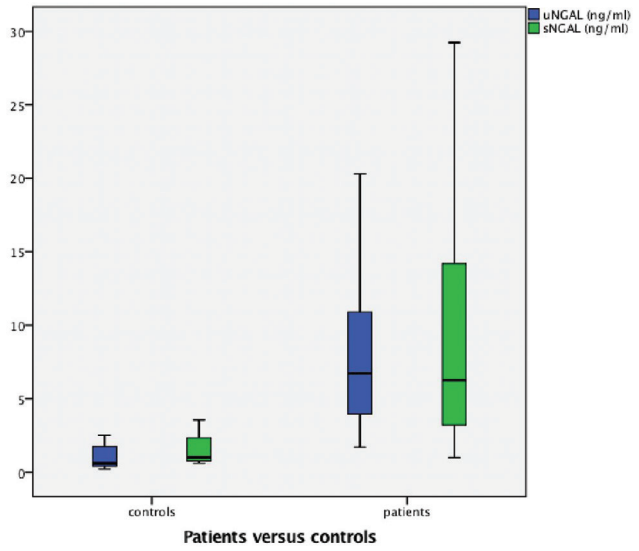

Figure 1: Comparison between control group and patient group regarding uNGAL and sNGAL. sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

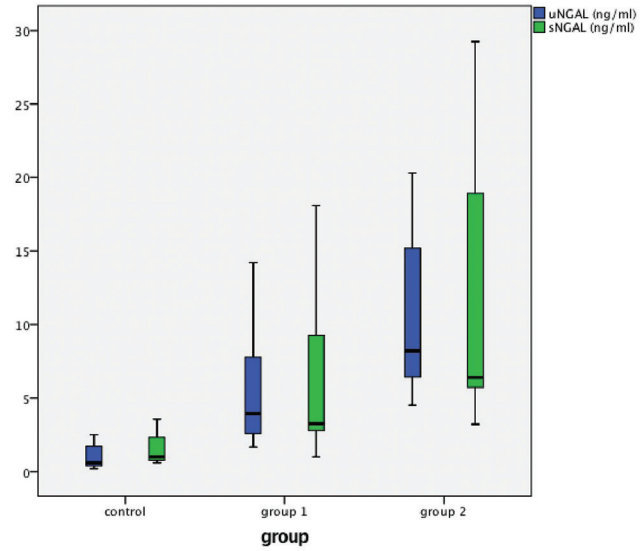

Figure 2: Comparison between patient groups 1 and 2 and control regarding uNGAL and sNGAL. sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

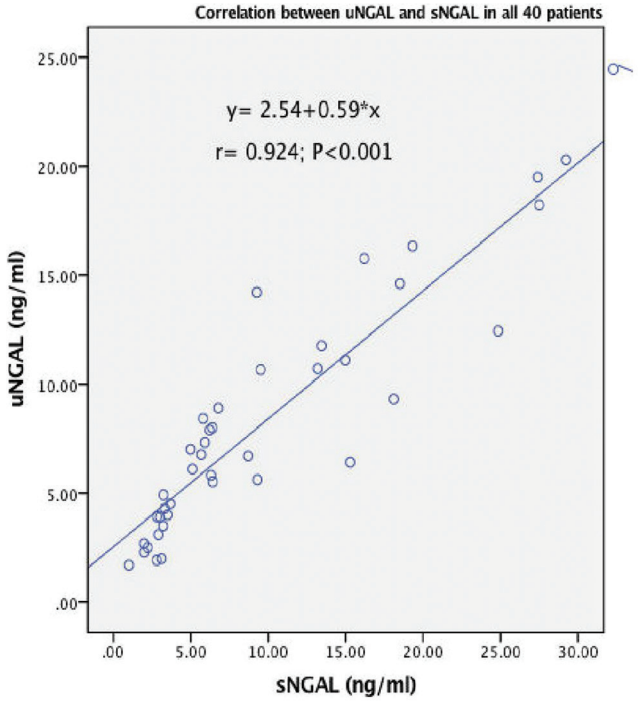

Figure 3: Correlation between sNGAL and uNGAL in patient group. sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

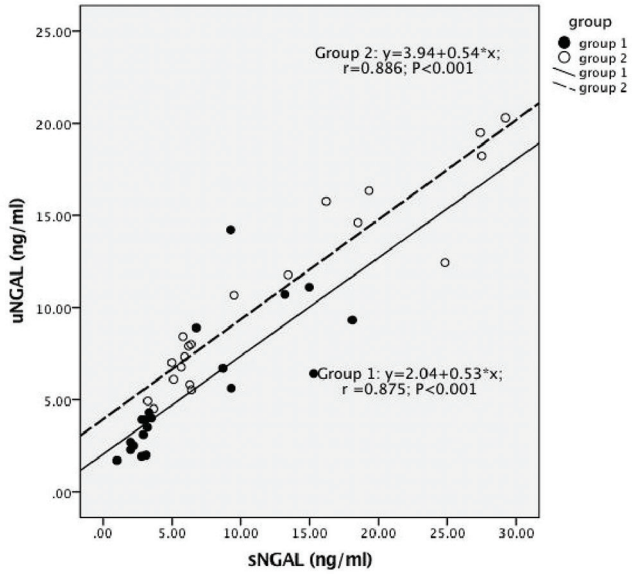

Figure 4: Correlation between sNGAL and uNGAL in groups 1 and 2. sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

and there were significant negative correlations with eGFR in group 1 ($P = 0.02$ and 0.033, respectively) (Figure 7).

On doing ROC analysis, the AUC for uNGAL, to identify patients with CKD was 0.98 [95% confidence interval (CI), 0.954–1.0, $P < 0.001$], with the best cutoff value of 2.2 ng/ml (sensitivity 92.5% and specificity 90%); the AUC for sNGAL was 0.937 (95% CI 0.88–0.994, $P < 0.001$), with the best cut-off value of 2.9 ng/ml (sensitivity and specificity 85%); and the AUC for CRP was 0.774 (95% CI 0.648–0.90), with the best cutoff value of 2.93 mg/dl (sensitivity 72.5% and specificity 60%, $P = 0.001$) (Table 6, Figure 8).

The AUC for the three markers to identify group 1 is very poor. The AUC for uNGAL to identify group 2 patients was 0.909 (95% CI 0.837–0.981, $P < 0.001$), with the best cutoff value of 5.72 ng/ml (sensitivity 85% and specificity 82.5%); the AUC for sNGAL was 0.876 (95% CI 0.79–0.963, $P < 0.001$), with the best cutoff value of 3.615 ng/ml

Table 1 Comparison between all patients and control groups

	Patient group		Control group		*P*
	Median	Range	Median	Range	
Creatinine (mg/dl)	1.5	0.85-3.1	0.8	0.5-1.02	<0.001
Urea (mg/dl)	33.85	18.3-49.6	18	13.2-24	<0.001
eGFR (ml/min/1.73 m^2)	59.5	30-87	107.5	80-128	<0.001
Alb/Cr ratio (mg/mmol)	48.1	6.4-215.7	1.21	0.82-2.11	<0.001
CRP (mg/l)	4.23	1.4-11.7	1.8	0.5-5.67	0.001
uNGAL (ng/ml)	6.73	1.7-20.3	0.6	0.21-2.51	<0.001
sNGAL (ng/ml)	6.25	1-29.23	1	0.6-3.56	<0.001

Alb/Cr, albumin/creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

Table 2 Comparison between groups 1 and 2 and control group

	Control		Group 1			Group 2			*P* (groups 1 and 2)
	Median	Range	Median	Range	*P*	Median	Range	*P*	
Creatinine (mg/dl)	0.8	0.5-1.02	1.21	0.85-1.59	<0.001	1.91	1.3-3.1	<0.001	<0.0001
Urea (mg/dl)	18	13.2-24	24.25	18.3-39.2	<0.001	38.35	25.4-49.6	<0.001	<0.0001
eGFR (ml/min/1.73 m^2)	107.5	80-128	65.5	61-87	<0.001	35	30-58	<0.001	<0.0001
Alb/Cr ratio (mg/mmol)	1.21	0.82-2.11	10.93	6.4-19.4	<0.001	152.4	76.8-215.7	<0.001	<0.001
CRP (mg/l)	1.8	0.5-5.67	2.84	1.4-5.22	0.25	6.53	3.98-11.7	<0.001	<0.001
uNGAL (ng/ml)	0.6	0.21-2.51	3.96	1.7-14.22	<0.001	8.21	4.51-20.3	<0.001	=0.001
sNGAL (ng/ml)	1	0.6-3.56	3.26	1-18.1	<0.001	6.4	3.23-29.23	<0.001	=0.006

Alb/Cr, albumin/creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

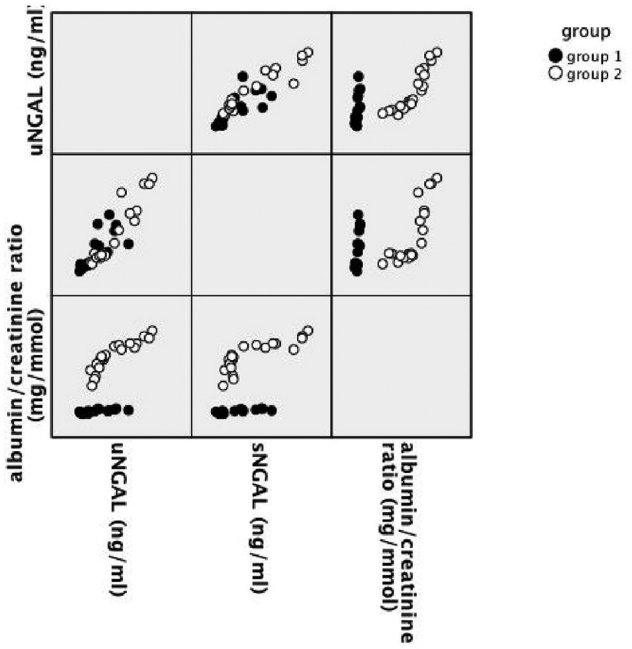

Figure 5: Correlation between uNGAL and sNGAL with Alb/Cr ratio in groups 1,2. Alb/Cr, albumin/creatinine; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

(sensitivity 95% and specificity 80%); and the AUC for CRP was 0.954 (95% CI 0.908–1.0), with the best cutoff value of

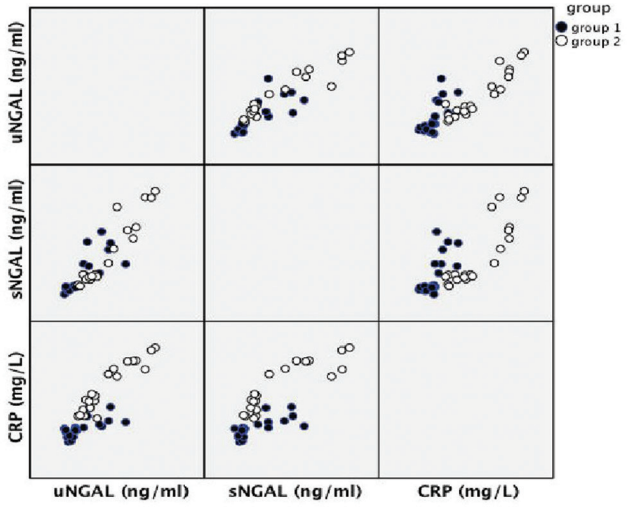

Figure 6: Correlation between uNGAL and sNGAL with CRP in groups 1 and 2. CRP, C-reactive protein.

4.1 mg/dl (sensitivity 95% and specificity 80%, $P < 0.001$) (Table 7, Figure 9).

Discussion

It is well known that patients with CKD frequently gain variable cardiovascular complication, which is the most popular reason of morbidity and mortality in end-stage renal disease. CKD has become a serious public health problem [12]. Globally, in

Table 3 Correlation between urinary neutrophil gelatinase-associated lipocalin and serum neutrophil gelatinase-associated lipocalin in all patient group

uNGAL	sNGAL		
	r	*P*	Significance
Cases groups	0.924	<0.001	HS
Group 1	0.875	<0.001	HS
Group 2	0.886	<0.001	HS

sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

Table 4 Correlation between urinary neutrophil gelatinase-associated lipocalin and serum neutrophil gelatinase-associated lipocalin in all patient group

	uNGAL		sNGAL	
	r	*P*	*r*	*P*
eGFR (ml/min/1.73 m^2)	−.754	<0.001	−0.646	<0.001
Alb/Cr ratio (mg/mmol)	0.828	<0.001	0.757	<0.001
CRP (mg/l)	0.842	<0.001	0.791	<0.001

Alb/Cr, albumin/creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

2017, 1.2 million (95% uncertainty interval 1.2–1.3) humans died owing to CKD [13].

According to KDOQI definitions [14], CKD diagnosis depends on enhanced albuminuria and diminished eGFR. However, there are several obstacles of being albuminuria as a marker of early disease. Some patients with kidney disease with decrease in glomerular filtration had no raised albuminuria more than 30 mg/g creatinine [15]. Moreover, albuminuria is not specific for CKD, and the hypertension or obesity often associated with type 2 diabetes mellitus can alter the glomerular filtration barrier causing elevated albuminuria [16]. The cutoff value of the urine Alb/Cr ratio more than 15 mg/g must be used to determine CKD, because it mostly predicts kidney disease-associated cardiovascular problems [17]. Meanwhile, reduced eGFR happens slowly after the kidney alterations in CKD, the early destruction being often associated with hyperfiltration [18]. However, in 30% of patients with type 2 diabetes mellitus having renal disease, changes in renal interstitium and tubules anticipate glomeruli injury [19].

The ideal urinary biomarker of tubular injury must be a noninvasive one, and therefore, assessment of a urinary protein

Table 5 Correlation between urinary neutrophil gelatinase-associated lipocalin and serum neutrophil gelatinase-associated lipocalin in groups 1 and 2

	Group 1				Group 2			
	uNGAL		sNGAL		uNGAL		sNGAL	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
eGFR (ml/min/1.73 m^2)	−0.506	0.02	−0.479	0.033	−0.79	<0.001	−0.659	0.002
Alb/Cr ratio (mg/mmol)	0.708	<0.001	0.749	<0.001	0.964	<0.001	0.839	<0.001
CRP (mg/l)	0.777	<0.001	0.784	<0.001	0.952	<0.001	0.909	<0.001

Alb/Cr, albumin/creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

Table 6 Diagnostic performance of cutoff value of urinary neutrophil gelatinase-associated lipocalin, serum neutrophil gelatinase-associated lipocalin and C-reactive protein in all patients

	AUC	95% CI	*P*	Sensitivity	Specificity	Cutoff value	Accuracy
uNGAL	0.98	0.954-1.0	<0.001	92.5	90	2.2	91.25
sNGAL	0.937	0.88-0.994	<0.001	85	85	2.9	85
CRP	0.774	0.648-0.9	0.001	72.5	60	2.93	66.25

AUC, area under curve; CRP, C-reactive protein; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

Table 7 Diagnostic performance of cutoff value of urinary neutrophil gelatinase-associated lipocalin, serum neutrophil gelatinase-associated lipocalin, and C-reactive protein in group 2

	AUC	95% CI	*P*	Sensitivity	Specificity	Cutoff value	Accuracy
uNGAL	0.909	0.837-0.981	<0.001	85	82.5	5.72	83.75
sNGAL	0.876	0.79-0.963	<0.001	95	80	3.651	87.5
CRP	0.954	0.908-1.0	<0.001	95	80	4.1	87.5

AUC, area under curve; CRP, C-reactive protein; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

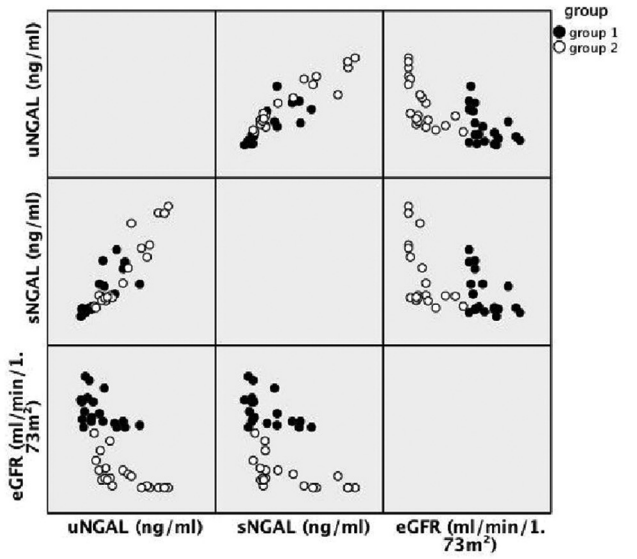

Figure 7: Correlation between uNGAL and sNGAL with eGFR in groups 1 and 2. eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

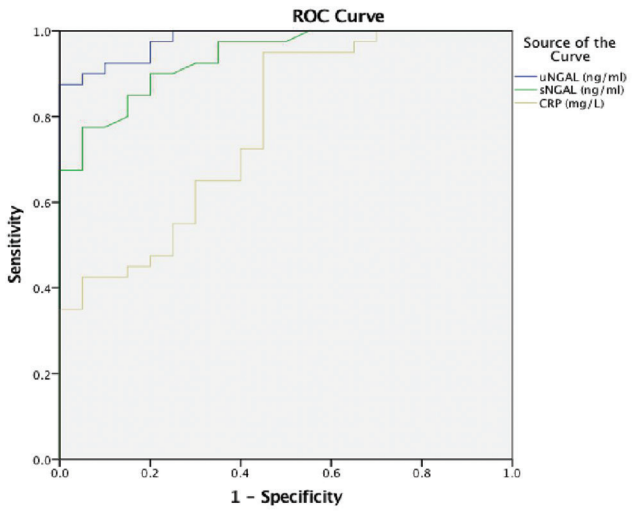

Figure 8: Receiver operating curve to predict CKD for uNGAL, sNGAL, and CRP. CKD, chronic kidney disease; CRP, C-reactive protein; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

immediately indicates injured tubular cells before settled tubule interstitial destruction occurs [20].

NGAL is found in many human tissues at very small concentrations. Kidney damage causes a rapid rise. It is easily excreted and detected in urine because of its small molecular size (25 kDa) and its resistance to degradation [21].

NGAL presents in three formulas: monomer, homodimer, and NGAL/MMP-9 complex [22]. The monomer form of NGAL is mainly released by tubular cells, whereas the homodimer one is mostly released from neutrophils [23]. So, different forms can be specific for variable CKD causes. The uNGAL consists of more complex forms, initially secreted from neutrophils and then excreted by the renal distal tubules epithelium [24]. It is well known that a urine sample is less stable than serum, owing to the effect of urinary output, timing of sampling collection, and storage temperature on it.

NGAL may be the best biomarker in CKD. It is originally present in activated neutrophils as an innate antibacterial defense, is released in huge amount from renal tubular cells following injurious stimuli, triggering specific iron-dependent pathways, besides the self–defensive aim to complement oxidative stress and cellular apoptosis. It was originally extracted from neutrophils initializing nephron development in embryonic kidney. It is one of the earliest, strongest developed genes and proteins in the tubular epithelium of the distal nephron and released from tubular epithelial cells after an inadequate blood supply to the kidney [21].

NGAL is an ideal biomarker of acute kidney injury (AKI). Tubular injury and systemic inflammation result in creating uNGAL in renal epithelia and neutrophils. We can use increased uNGAL to anticipate AKI [25], differentiate between intrinsic AKI from pre-renal AKI [26], anticipate renal non-recovery [27], CKD progression for a prolonged time, end-stage renal disease, and in-hospital mortality [28]. The report by Lumlertgul *et al*. [29] demonstrated an association between uNGAL and constant AKI.

However, studies also suggest a possible role of NGAL in CKD. NGAL as a biomarker in CKD would represent the ongoing process of renal damage rather than a simple marker of lost function as serum creatinine and useful for early diagnosis and prediction of renal diseases [30]. In an experimental study in rats, ongoing inflammation and immune activity were found to be involved with the pathogenesis of CKD, and NGAL was upregulated, suggesting that it may be a valuable biomarker for the development of CKD after AKI [31]. NGAL has recently been proven useful to quantitate CKD. Thus, there has been interest in NGAL as an additional measure of kidney impairment in CKD [32]. The study by Guo *et al*. [33] was the first one to evaluate the prognostic value of sNGAL in older patients with CKD. They showed that NGAL levels are independently associated with eGFR and renal-disease-related clinical parameters, predicting renal function decline.

Our results showed a highly significant statistical increase in serum and urine levels of NGAL in the early renal impaired patients compared with the control group. On comparing patient groups 1 and 2 with controls, there was also significant elevated sNGAL and uNGAL and a significant increase in sNGAL and uNGAL between the two patient groups. These results are in accordance with that of the study by Patel *et al*. [30], which reported that the sNGAL and uNGAL values are correlated with the severity of CKD and anticipate its progression. The underlying mechanism of connection between uNGAL and sUGAL with early renal disease was supported by earlier

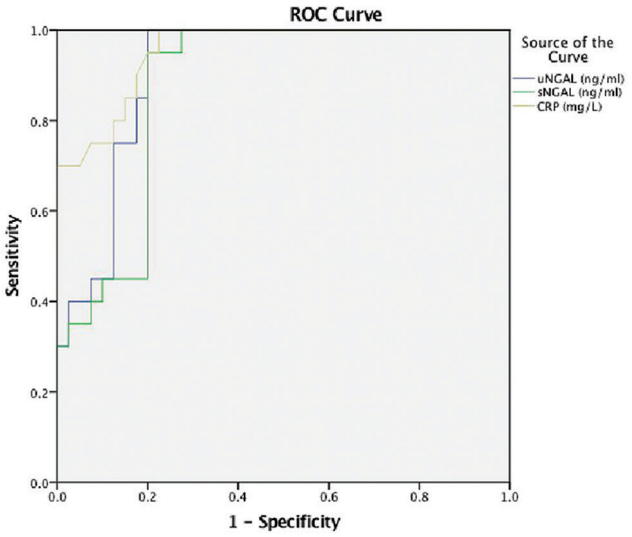

Figure 9: Receiver operating curve to predict CKD stage 2 for uNGAL, sNGAL, and CRP. CKD, chronic kidney disease; CRP, C-reactive protein; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urinary neutrophil gelatinase-associated lipocalin.

studies, applied on patients with early-stage CKD of different causes and control and confirmed that NGAL discriminates renal impairment and is a powerful and independent risk factor for progression. High uNGAL found in kidney diseases is not only owing to passive loss through glomerular membrane, but also tubular cell secretion owing to its injury. So, increased uNGAL may be a 'real-time' marker of how much destruction and active stress is found in the CKD [34]. On the contrary, Liu *et al*. [35] confirmed that uNGAL magnitudes do not anticipate CKD progression. Another study on type 2 diabetic patients showed that uNGAL was not significantly elevated in the early stage of renal impairment [36].

In Poland, the report by Żyłka *et al*. [37] confirmed that patients with diabetic nephropathy had raised NGAL magnitudes. Moreover, patients with higher baseline NGAL showed a considerably increased risk of worsening residual renal function within 1 year, compared with those with lower baseline values. This attributes to NGAL an interesting predictive value and may help in early assessment of kidney disease in patients with type 2 diabetes mellitus. The study by George and Gounden [38] stated that patients with CKD had significantly raised sNGAL levels, confirming its responsibility as an early marker of kidney injury.

The importance of NGAL in the pathogenesis of CKD was stated by Kubben *et al*. [39] who reported that NGAL forms a compound with MMP-9, protecting the latter from degeneration and so protecting MMP-9 enzymatic action which is involved in renal disease. This is in agreement with the report of Guo *et al*. [33], which stated that the tubular injury marker NGAL is indicator for disease activity and renal function because tubular epithelial cells are important in the pathogenesis of CKD progression. In addition, Malyszko *et al*. [40] stated that tubular epithelial cells secrete huge concentrations of NGAL directly following traumatic stimuli. NGAL is recognized in serum and urine before creatinine rise. Early detection and treatment in patients with CKD may be easier with observing NGAL.

In our study, there was a highly significant increase between the patient group and control group regarding Alb/Cr ratio, with a significant increase between patient groups 1 and 2 (early-stage CKD), which is in agreement with the study by Żyłka *et al*. [37], revealing a significant difference in Alb/Cr ratio between patients with early-stage diabetic kidney disease and control. It is well known that albuminuria is a predictor for renal progression, but there are some patients having diminished GFR, and to some extent normal albuminuria. uNGAL assessment may help in determination of these patients [41].

There was a significant positive correlation of uNGAL and sNGAL with Alb/Cr ratio in the patient group. This is in accordance with a study done by Żyłka *et al*. [37], who reported that a direct correlation of Alb/Cr ratio with uNGAL and sNGAL levels, indicating NGAL to be a marker of renal function. Bolignano *et al*. [42] also confirmed elevated uNGAL concentrations and highly associated with proteinuria in a huge number of patients with proteinuria. However, the results of Coppolino *et al*. [43] contradict ours. They did not find any correlation between uNGAL and proteinuria, may be owing to small sample size and different causes of their glomerular disease.

Our study showed a significant negative correlation of uNGAL and sNGAL with eGFR. This agrees with many studies in patients with CKD which confirmed the negative association of uNGAL and sNGAL levels with GFR. A reduced GFR was correlated with increased uNGAL and sNGAL concentrations in patients with CKD, indicating that it may assess the remaining GFR more actually than the creatinine magnitude [32,33] In the contrary, Bhavsar *et al*. [44] showed no correlation between uNGAL concentration and GFR on patients with type 1 diabetes.

We found a significant positive correlation between sNGAL and serum creatinine in patients with CKD (stages 2–3). These findings are in accordance with Bolignano *et al*. [42], who revealed that sNGAL is formed following renal damage, then glomerular filtration and tubular uptake, and it is secreted by the injured tubules. Moreover, Bolignano *et al*. [45] with other group confirmed independent and inverse correlation of sNGAL and uNGAL with GFR in patients with early-stage CKD, signifying that they estimated CKD progression independent of GFR. Meanwhile, Garlo *et al*. [46] stated that there was no significant correlation between uNGAL and serum creatinine in patients, and uNGAL is suggestive of a decreased GFR without high creatinine level.

The sustained secretion of NGAL by injured tubular cells leads to elevated blood, urine, and kidney NGAL concentrations during CKD (the so-called forest fire hypothesis), whereas the serum creatinine rise and GFR reduction are passive results of diminution of normal cells or nephrons. Therefore, NGAL

may be an actual biomarker of existing active kidney damage in CKD [34]. Recently, the study by Bennett *et al*. [47] showed that NGAL is able to distinguish between steroid-sensitive and steroid-resistant idiopathic nephrotic syndrome children.

More recent, the paper by Guo *et al*. [33] confirmed the correlation between NGAL and anemia. Anemia and raised NGAL levels are found in CKD, and also in chronic inflammation and cancer; this may indicate the relation between anemia and NGAL. Devireddy *et al*. [48] found that NGAL causes apoptosis and hinders erythroid progenitor cell differentiation *in vitro* culture. So, NGAL plays an important part in anemia.

NGAL is not only a marker of CKD progression but also an inflammatory marker [49]. It is released by circulating neutrophils. Inflammation and reactive oxygen species play important roles in the malnutrition of CKD [50].

Regarding CRP, in this study, there was a highly significant increase between the patient group and stage 3 CKD (group 2) compared with control group. This finding is confirmed by the study by Żyłka *et al*. [37] on 80 diabetic patients with nephropathy.

Regarding the diagnostic performance of uNGAL in all patients with CKD compared with the control group, the AUC was 0.98; the best diagnostic cutoff was 2.2 ng/ml, with diagnostic sensitivity of 92.5%, specificity 90%, and 91.25% accuracy. For the diagnostic performance of sNGAL (ng/ml) in patients with CKD, the AUC was 0.937; the best diagnostic cut-off was 2.9 ng/ml, with diagnostic sensitivity of 85%, specificity 85%, and 85% accuracy. Guo *et al*. [33] showed sNGAL ROC curve had sensitivity of 92.3%, a specificity of 75.2%, and AUC of 0.897 in predicting progression of CKD [33]. Patel *et al*. [30] and Coppolino *et al*. [43] revealed AUC of ROC analysis, for uNGAL, for predicting worsening renal function was 0.778 and 0.76 with a sensitivity 73.08 and 80.9%, specificity of 71.43 and 67.5%, respectively.

Regarding the AUC for uNGAL in our study, to identify patients with stage 3 CKD (group 2), it was 0.909, with the best cutoff value of 5.72 ng/ml (sensitivity 85% and specificity 82.5%). The AUC for sNGAL was 0.876, with the best cut-off value of 3.615 ng/ml (sensitivity 95% and specificity 80%). The paper by Guo *et al*. [33] revealed that AUC of ROC analysis, for uNGAL, for stage 3 CKD group was 0.869, with a sensitivity and specificity of 89.7 and 72.6%, respectively.

In addition to that NGAL is a biomarker of renal damage, it may be a biomarker of kidney function after transplant [51]. Renal injury is mainly owing to ischemia and reperfusion injury in transplant. NGAL may show the degree of injury after reperfusion, and the possibility of improvement of kidney function. NGAL was an early predictor of delayed graft function after kidney transplant when estimated 24 h after transplantation with an AUC-ROC of 0.82 [52]. Haase-Fielitz *et al*. [53] showed that measuring sNGAL and uNGAL 6–12 h after transplantation may anticipate delayed graft function with 82% sensitivity and specificity.

Our study has several limitations. It was an observation study, sNGAL and uNGAL were measured only at baseline, follow-up studies should be performed at every stage for further risk assessment, it was a single-center study, and the number of patients was relatively low.

Conclusion

This study indicates that NGAL as a noninvasive marker may help in the assessment of early CKD. However, more multicenter studies are needed to further investigate the accuracy of NGAL on a larger population and to determine whether therapeutic measures targeting NGAL balance would be helpful in delaying the progression of CKD and complications.

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Conflicts of interest

None declared.

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