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RESEARCH ARTICLE

Role of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Patients with Chronic Kidney Disease (CKD)

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Abstract: CKD is defined as an ongoing illness, characterized by distinguishing features in its developments in the structure or functions associated with several causes. It affects nearly 10% of the world's population, with deaths exceeding 1.2 million on an average annual rate, thereby increasing the demand for authentic biomarkers. To analyze the levels of serum (NGAL) in CKD patients in Iraq, its diagnostic value, correlations, and relationship with biomarkers for renal functions, demographics, and other variables. A case-control study with 88 participants, consisting of 30 dialysis patients, 30 pre-dialysis patients, and 28 controls, was undertaken. ELISA kits were used to examine levels of Serum NGAL. Median value for NGAL in CKD is 117.5 ng/mL, with interquartile range values ranging from 40 to 597, with IQR: 107. It has significant increase ($p < 0.001$) compared to controls (median value 59.5 ng/mL, with interquartile range from 33 to 255, IQR: 73). It has Area Under Curve value (AUC = 0.748) ($p < 0.001$) with sensitivity at 68.5 ng/mL with sensitivity of 73% specificity of 64% in ROC curve analysis. It has highly negative relationship with eGFR ($R = -0.654$, $p < 0.001$). It has moderate positivity with Creatinine levels in blood ($R = 0.259$, $p = 0.046$). High levels of NGAL in CKD patients reveal its promising role in CKD, whereby its biomarker character is non-invasive for CKD detection. Its large magnitude of negative association with eGFR reveals its role in estimating reductions in renal functions, being an efficient biomarker in CKD progression. Lastly, there were no significant variations in levels of NGAL with demographics.

Keywords: Chronic kidney disease, Estimated glomerular filtration rate, Serum creatinine, ROC, Neutrophil gelatinase-associated lipocal

INTRODUCTION

Chronic Kidney Disease (CKD) is a disorder characterized by changes in renal function and structure resulting from various factors. Generally, it is defined as eGFR below 60 mL/min per 1.73 m², a deterioration in kidney function, or damage, such as identified abnormalities, haematuria, or albuminuria (1). The worldwide burden of CKD is significant and increasing: 10% (approximately) of adults globally are impacted by some form of CKD, leading to 1-2 million fatalities (2). CKD is anticipated to rank as the fifth-highest cause of mortality worldwide in 2040, representing one of the major causes of mortality (3). The spread of various aetiologies of CKD exhibits significant regional variation. Several aetiologies contribute to the development of CKD, such as prevalent and extensively studied conditions including glomerulonephritis, hypertension, diabetes, and cystic kidney disorders; yet, the etiology of chronic kidney disease remains incompletely elucidated (4).

As a member of the lipocalin protein family is neutrophil gelatinase–associated lipocalin (NGAL). 8 b-strands that combine to form a b-barrel that encloses a calyx in lipocalins. The calyx binds and transports low-molecular-weight chemicals that interact with cell-surface receptors (5). A study indicated that plasma NGAL is a significant marker of interstitial lesions in CKD patients. Researchers have evaluated the final stage of CKD risk levels utilizing NGAL. Evidence suggests that NGAL may work as a mediator in the course of CKD (6).

It was found that NGAL was produced by tubular epithelial cells in the distal nephron after tissue damage, such as ischemic renal injury, and that it was among the highly activated proteins and genes in these cells. Additional research has revealed that urine contains multiple molecular forms of NGAL. This includes a monomeric form produced by kidney tubular epithelial cells and a dimeric form made by neutrophils (7). In patients with early-stage CKD, Plasma NGAL has been associated with changes in eGFR. Currently, markers with low sensitivity that indicate the risk of fast disorder progression in individuals with CKD are time-consuming and expensive to evaluate. Therefore, there is a need for novel, easy-to-measure indicators capable of predicting its progression, either independently or in conjunction with traditional risk markers.

In the current study, the possibility of using indicators of tubular injury to predict deterioration in kidney function was investigated. New prognostic markers must be developed, either alone or in combination with conventional risk markers, to predict the rate of disease progression in CKD. Measuring kidney damage markers such as (NGAL), along with the inflammation, is of significant interest. This biomarker is particularly valuable due to its cost-effectiveness and ease of use.

This article sets certain goals to achieve: Assessment of serum levels of (NGAL) in Iraqi people with CKD and compare them with a control group. Additionally, study the possibility of utilizing the (NGAL) biomarker as a new diagnostic biomarker in CKD to measure the correlation among NGAL serum levels with Creatinine and Urea, and demographic features.

MATERIALS AND METHODS

Study Design: This work is a case-control study conducted on 88 Iraqi participants of average age (40-80) years from November 2024 to March 2025. Participants were enrolled from two locations: Imam Husayn Hospital and Habib Bin Mudhahir Kidney Dialysis Center. A questionnaire was used to collect patient data, including age, sex, body mass index (BMI), family history of CKD and other diseases, Diabetes status, blood pressure, and other relevant medical information.

Patients: Current work was conducted on 60 patients with CKD (30 males and 30 females) of average age (40-80). They were diagnosed by a Consultant physician, based on their clinical signs, symptoms and laboratory tests (Urea, Creatinine, and eGFR).

Blood Samples:
Five milliliters of venous blood was obtained from patients reporting to the nominated centers. The blood was placed in a plain gel tube and left to clot for about 30 minutes at room temperature. Later, serum separation was conducted by centrifugation at 3000 x g for 5 minutes. The serum was aliquotted into three Eppendorf tubes and stored at -80 °C in a deep freezer prior to analysis, to reduce frequent thawing and freezing.

Biochemical Analysis:
The serum NGAL was analyzed by single tube assay by enzyme-linked immunosorbent assay. The serum biochemical values, such as serum urea, and serum creatinine, were determined in each of two Eppendorf tubes. The values of serum urea and serum creatinine were determined by analyzing with a chemical auto-analyzer, depending upon automated sampling, reagent reconstitution, and colorimetry.

Inclusion Criteria:
Patients with CKD were diagnosed according to the medical history, clinical examination, and serum (Urea, Creatinine, and eGFR).

Exclusion Criteria:
Cancers , Acute kidney injury, Patients with COVID-19, Accident cases.

Statistical Analysis: SPSS tool (IBM SPSS 26.0) has been used for statistical analyses. Data normality was tested using the Shapiro-Wilk; nonparametric tests were used. For non-normal data, the following were applied: Mann-Whitney U, Kruskal-Wallis, and Spearman correlation. To estimate odds ratios with 95% CI, logistic regression was used. Furthermore, ROC analysis identified optimal diagnostic thresholds. A p-value less than 0.05 was regarded as significant.

Ethical Approval: It is granted by the Ethical Committee of the College of Medicine/University of Karbala and the Karbala Health Directorate. Consent was obtained from participants after clarifying the nature of the study and its objectives to each Participant. 25-89, 26/10/2025.

RESULTS AND OBSERVATIONS:

Table 1 offers an insight into the comparison of various socio-demographic parameters among three groups of patients, those undergoing dialysis, those at the pre-dialysis stage, and those belonging to the control group. As far as the aspect of aging is concerned, it was revealed that the Control group was relatively younger, with 80% of subjects belonging to this group aged below 60 years. The group of patients undergoing dialysis included the largest share of older patients, with 16.7% above 70 years, while those at the pre-dialysis stage included patients moderately distributed, with those above 70 years forming 30% of this group.

The distribution by sex was equivalent in all groups, with roughly equal male and female participants. This balances the potential influence of gender-generated variables in testing subsequent analyses of clinical and/or biochemical parameters. The BMI analysis revealed that obesity was most predominant in the Control group with 50% obesity, and in those on dialysis at 40%. The opposite was seen with respect to those with normal BMI, with the highest incidence found in those in the pre-dialysis group at 63.3%.

Table 1 Socio-Demographic Characteristics

Characteristics		Groups		
		On-dialysis n=30	**Pre-dialysis** n=30	**Control** n=28
Age	<40 years	5 (16.7%)	0	9 (32.1%)
	40-50 years	7 (23.3%)	4 (13.3%)	13 (46.4%)
	51-60 years	4 (13.3%)	7 (23.3%)	5 (17.9%)
	61-70 years	9 (30.0%)	10 (33.3%)	1 (3.6%)
	>70 years	5 (16.7%)	9 (30.0%)	0
Sex	Male	14 (46.7%)	15 (50.0%)	14 (50.0%)
	Female	16 (53.3%)	15 (50.0%)	14 (50.0%)
BMI	Normal weight	12 (40.0%)	19 (63.3%)	4 (14.3%)
	Overweight	6 (20.0%)	8 (26.7%)	11 (39.3%)
	Obese	12 (40.0%)	3 (10.0%)	13 (46.4%)

Table 2 compares NGAL levels between patients with CKD (n=60) and controls (n=28), revealing significant elevations in NGAL levels in the CKD group. Median NGAL levels were 117.5 ng/mL in CKD patients, with a range of 40–597 and an IQR of 107. This was higher than the control group (median 59.5 ng/mL, range 33–255, IQR: 73). The difference was significant ($p < 0.001$).

Table 2: NGAL Levels in CKD and control group patients.

Biomarker	Control n=28		Patients n=60		P value
	Median (Min-max)	**IQR**	**Median (Min-max)**	**IQR**	
NGAL ng/ml	**59.5 (33-255)**	**73**	**117.5 (40-597)**	**107**	**<0.001**

Mann-Whitney Test was significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum.

Table 3 presents a comparative analysis of NGAL levels across three groups (Control, Pre-dialysis, and On-dialysis), demonstrating a progressive increase in NGAL levels with advancing CKD stages. NGAL, median levels rose from 59.5 ng/mL in the Control group (range: 33–255; IQR: 73) to 108.5 ng/mL in Pre-dialysis patients (range: 40–320; IQR: 102), and further to 135 ng/mL in On-dialysis patients (range: 44–597; IQR: 145).

Table 3 NGAL Levels between Sub-Groups (pre, on dialysis and control).

Biomarker	Control n=28		Pre-dialysis stage n=30		On dialysis stage n=30		P value
	Median (Min-max)	**IQR**	**Median (Min-max)**	**IQR**	**Median (Min-max)**	**IQR**	
NGAL ng/ml	59.5 (33-255)	73	108.5 (40-320)	102	135 (44-597)	145	<0.001

Kruskal-Wallis Test was significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum.

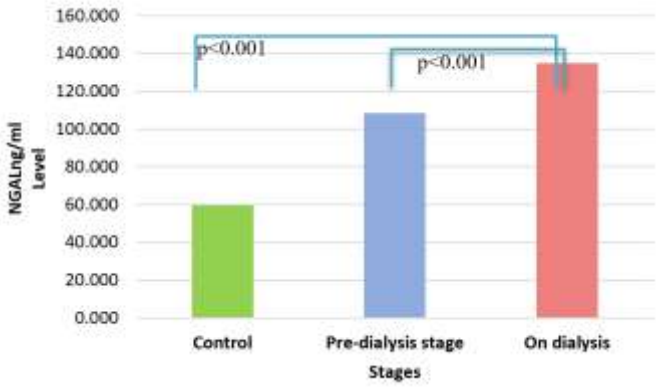

Fig. 1: Comparison of NGAL Levels in the study group and sub-groups.

Table 4 assesses the correlation between kidney injury biomarker NGAL and standard renal function markers (Urea, Creatinine, and eGFR) in CKD patients, using Spearman's rank correlation coefficients. With Urea, there was a weak positive correlation in NGAL (R = 0.051, p = 0.697), with neither reaching statistical significance. With Creatinine, a moderate positive correlation with NGAL was observed (R = 0.259, p = 0.046), indicating a statistically significant correlation. With eGFR, NGAL also demonstrated a strong negative correlation (R = -0.654, p < 0.001), which was highly statistically significant.

Table 4: Correlation of NGAL with (Urea, Creatinine, and eGFR) in CKD Patients.

Biomarker	Urea		Creatinine		eGFR	
	R	P-value	R	P-value	R	P-value
NGAL ng/ml	0.051	0.697	0.259	0.046	-0.654	<0.001

Spearman Rank Test; *Correlation is significant at the 0.05 level; + = positive; R: Spearman correlation coefficients.

Table 5: ROC Curve: NGAL this table analyses the diagnostic accuracy of NGAL to discriminate CKD subjects from controls by means of the ROC curve. It is observed in Table 4 that NGAL has a diagnostic accuracy with Area Under Curve (AUC) value of 0.748, which is statistically significant (p < 0.001). It is observed that at an optimally chosen cutoff point of 68.5 ng/mL, NGAL's specificity is 64% with sensitivity of 73%, meaning its clinical efficiency in excluding CKD is more than its efficiency in identifying CKD.

Table 5: AUC, Optimal Cut-off, Sensitivity, and Specificity of NAGL Based on ROC Analysis for Patients with Control Groups

NGAL ng/ml	AUC	P-value	Cut off ng/ml	Sensitivity	Specificity
Control–Patients	0.748	<0.001	68.5	73 %	64 %

ROC: significant at p ≤ 0.05.

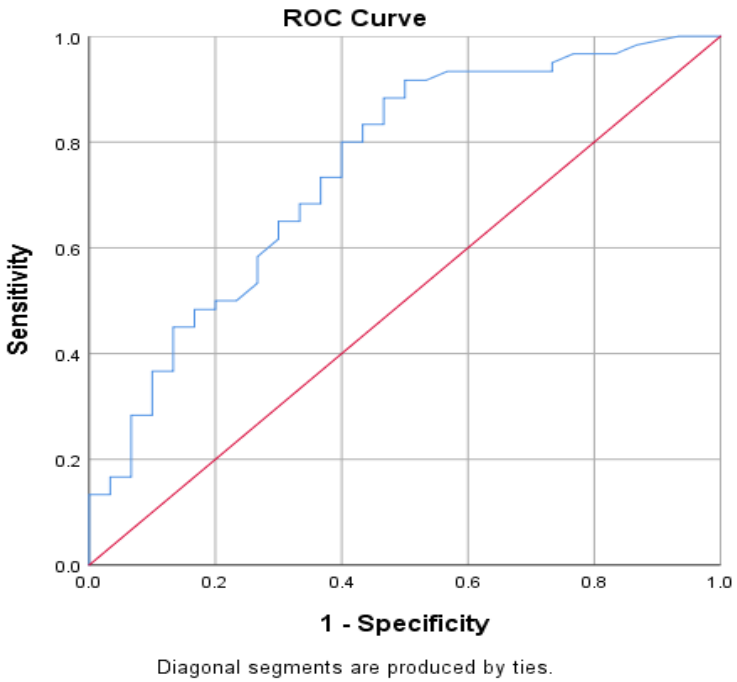

Fig. 2: ROC curve for NAGL in CKD patients compared to the control group.

DISCUSSION

Table 1 above shows the demographical disparities seen in all cohorts, which is imperative in understanding all subsequent clinical and biomarker results. The young demographic dominance seen in the Control group, as well as the high proportion of older individuals in those on dialysis, represents, of course, the established relationship between older ages and disease progression of chronic kidney disease (CKD). The intermediate demographic seen in the pre-dialysis group, however, establishes earlier stages along the disease process chronology. Furthermore, with all groups having nearly equal demographics by gender, all potential aspects concerning differential outcomes by gender can be ruled out as confounders. Finally, and perhaps more importantly, while healthy individuals predominantly shown are obese, those in the pre-dialysis group regress towards having normal body mass index, which has been established to be due to weight loss in CKD patients, and has been explained by chronic inflammation, metabolic disturbances, and reduction in food nutritional value, all three culprits noted as promoted by chronic kidney disease complications (8,9,10).

The significant elevation of neutrophil gelatinase-associated lipocalin (NGAL) was found in CKD patients, as shown by Table 2, when its level was compared with that of control patients. This difference has statistical significance, indicating that it is not likely due to mere chance. This is in agreement with existing findings. A case-control study conducted at Wasit Province, Iraq, in 2024 found that severe CKD stages 3-5 was significantly correlated with increased serum concentrations of NGAL at 1,195.3 ± 695.5 ng/mL, with $p < 001$, as opposed to stages 1-2 CKD at 366.1 ± 261.5 ng/mL, with $p < .001$, and an OR = 56.9, 95% confidence interval = 9.48-343.1, $p < .001$ (11). The elevation of NGAL in CKD patients can be used as an early indication of tubular damage, whose cells, damaged.

As illustrated in Table 3, compared to the control group, this study demonstrates a statistically significant increase in serum NGAL levels in CKD patients (before and during dialysis). Significantly, NGAL levels were elevated in the dialysis group compared to the pre-dialysis and control groups. The progressive increase suggests a strong correlation between kidney function deterioration and NGAL elevation. This supports its utility as a sensitive biomarker of tubular injury and renal stress. NGAL is known to be unregulated in response to ischemic and nephrotoxic insults. This makes it valuable and an early indicator of CKD (14), (15).

NGAL demonstrated a weak positive correlation with serum creatinine, and a strong negative correlation with eGFR, showing no significant correlation with urea levels (see Table 4). These findings align with earlier research, as prospective studies .Indicated a negative correlation between eGFR and kidney failure relative to the control group, alongside elevated NGAL levels. NGAL was more closely linked to creatinine, suggesting that it might be able to detect early tubular injury. The lack of a significant correlation between NGAL and urea may be due to the limited specificity of serum urea as a marker of renal function (16), (17).

NGAL had a large area under the receiver operating characteristic curve with an optimal cutoff point, indicating high sensitivity with moderate specificity (Table 5). It appears to have good accuracy in distinguishing CKD subjects from controls, indicating detection not only of tubular damage in CKD but also inflammatory changes. These data agree with previous reports on the diagnostic accuracy of NGAL in CKD On clinical grounds, these findings verify its value in early detection, while on scientific grounds, it again proves its role as a validated biomarker. More recently, evidence by Li et al. in 2024 validated its high accuracy in CKD (18), (19).

CONCLUSION

Evidence shows that there is a significant increase in NGAL levels in CKD patients compared to those in the control group. Accordingly, being non-invasive, NGAL has promising applications for diagnostic, progression, as well as improving risk stratification in CKD. NGAL had a weak positive relationship with creatinine levels, while its relationship with eGFR was strongly negative, indicating no relationship with urea reabsorption levels. Accordingly, based on eGFR, it could be concluded that there is an inverse relationship between NGAL levels, indicating their progression in CKD, thereby solidifying their status as CKD progression biomarkers. Amongst demographic variables, there have been no statistically significant variations in levels of NGAL.

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