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NGAL-A Promising Biomarker for AKI in Severe COVID-19 Disease and Its Prognostic Role: A Prospective Study

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Abstract

Background: COVID-19 disease has stunned the world with its relentless spread and high mortality rates. Acute kidney injury has been an important consequence of the disease, contributing to mortality. There is unavailability of data for using neutrophil gelatinase associated lipocalin as a biomarker for acute kidney injury in patients with severe COVID-19 disease.

Aim: To evaluate the role of inflammatory marker neutrophil gelatinase associated lipocalin in severe COVID-19 disease.

Methods: A prospective observational study was conducted at various tertiary care hospitals in Prayagraj from January 1st 2021 to March 15th 2021, which included only patients with severe COVID-19 disease requiring ICU admission. Patients of known renal diseases were excluded from the study. The study was done on 110 patients, out of which 5 patients were excluded. Blood as well as urinary samples for neutrophil gelatinase associated lipocalin and the other laboratory parameters were collected within 8 hours of admission. The patients were followed until the hospital stay, and investigations were repeated every 3 days. The primary outcome was development of acute kidney injury, while secondary outcomes included-need of renal replacement therapy such as hemodialysis, mortality. Patients who developed renal dysfunction were noted as our cases and the others were noted as controls.

Results: The study was done on 105 patients, out of which 60 developed AKI (Acute kidney injury) and were noted as cases while 45 were our controls. The cutoffs for serum and urinary neutrophil gelatinase associated lipocalin for predicting acute kidney injury were found to be >91 and >80.6 respectively (p value<0.001). Serum and urinary NGAL had correlation coefficient of 0.462 and 0.380 with p value 0.005 and 0.02 respectively. Serum and urinary neutrophil gelatinase associated lipocalin showed significant predictive values for requirement of hemodialysis (renal replacement therapy) with p-value of 0.007 and 0.003 respectively, using multivariate analysis. Serum and urinary neutrophil gelatinase associated lipocalin at values >136.5 and >131 respectively were found to predict mortality (p value<0.001).

Conclusion: Neutrophil gelatinase associated lipocalin correlates strongly with development of acute kidney injury and the various radiological changes. Thus, it can be used as a prognostic marker in severe COVID-19 disease.

Keywords

NGAL • AKI • COVID-19 • Prognosis • Mortality • Hemodialysis

Introduction

With the spread and advent of SARS-CoV-2, health care systems around the globe faced a new challenge managing the disease with significant morbidity and mortality [1].

COVID-19 is mainly a respiratory tract infection which causes cytokine storm and leads to hyperimmune phase causing severe disease and ARDS. The most common reported reasons for intensive care unit admission for patients with severe coronavirus disease 2019 (COVID-19) are either hypoxemic respiratory failure leading to mechanical ventilation or hypotension requiring vasopressor support [2].

Patients with severe COVID-19 disease often develop a hyperinflammatory response with high levels of C-Reactive Protein (CRP) and excessive production of inflammatory cytokines, including Interleukin (IL)-1, IL-6, IL-8, IL-10, TNF, GM-CSF and IFNγ. This detrimental host response leads to sepsis and eventually septic shock, which aggravates renal dysf unction [3].

AKI has emerged to be an important consequence of COVID-19, with rates as high as 33%–43% among hospitalized patients [4] and upto 50% [5] in few studies. AKI requiring RRT has been reported in upto 20% patients and is associated with a hospital mortality rate of >60% [2]. Owing to the high incidence and mortality seen in patients with AKI, arises the need of an early marker for quick recognition and management of patients with AKI.

In the recent years, Neutrophil Gelatinase Associated Lipocalin (NGAL) has proven itself as a potential marker and has been said to be the troponin of renal diseases. It has been studied as a potential biomarker in various diseases such as-development of AKI after cardiac surgery [6], for nephropathy in sickle cell anemia [7], post Cardiopulmonary Bypass [8], for contrast induced nephropathy [9], following ECMO (Extra Corporeal Membrane Oxygenation) [10].

However, there is no data on the utility of NGAL as a biomarker in patients with AKI in COVID-19. Therefore, this study is an attempt to determine the predictability of AKI in COVID-19 patients using NGAL as a biomarker.

Materials and Methods

This study was designed as a prospective cohort analysis in 105 patients with severe COVID-19 disease, admitted in COVID ICU from 1st January 2021 to 15th March, 2021 at SRN Hospital, Prayagraj, Balaji Hospital, Prayagraj, Phoenix Hospital, Prayagraj. The patients were followed during the hospital stay and their outcomes were noted. Inclusion criteria comprised of age >18 years (male or female), severe COVID-19 disease (characterized by hypoxia–SpO2<90% on room air or respiratory

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rate >30/min), while patients with raised baseline serum creatinine >1.3 mg/dl prior to admission or eGFR<90 ml/min, patients of CKD and on Renal Replacement Therapy and those unwilling for study related diagnostic procedures were excluded. After obtaining ethical committee clearance and informed consent, clinical data and laboratory investigations were collected and noted.

Baseline serum creatinine was defined as the steady state level of creatinine 4 weeks before admission. If not available, the admission value or the lowest serum creatinine during the hospital stay was used as a surrogate baseline. Blood samples for NGAL were collected within 8 h of admission to ICU aseptically via venipuncture. The first urine of the day (mid-stream) was collected aseptically into a sterile container and tested for urinary NGAL. NGAL was tested by ELISA kit-ELABSCIENCE® using the sandwich ELISA principle. Serum urea and serum creatinine and other laboratory parameters were measured for three consecutive days, or for the duration of hospital stay, whichever was later. Patients developing AKI during hospital stay were noted and defined as our cases, while the patients who did not develop AKI were our controls. Staging of AKI was done using AKIN criteria. The primary outcome (development of AKI) and the secondary outcomes (mortality, need of Renal Replacement Therapy (RRT), radiological changes and its severity) were noted.

Statistical analyses

The quantitative data is expressed as mean ± SD. Categorical variables are expressed in number and percentages. Correlation of various parameters is calculated using Spearman's rho correlation coefficient. Receiver Operating Characteristic (ROC) curves are drawn, and the Area Under the Curve (AUC) was calculated to find the cut-off points and calculate the threshold specificity, sensitivity and diagnostic accuracy for predicting AKI and mortality outcomes. All statistical analyses are conducted using SPSS version 23.

Results

A total of 110 COVID-19 patients falling in sampling frame were enrolled in the study, out of which 5 patients were excluded owing to the exclusion criteria (2 patients were newly diagnosed to have CKD while 3 had raised baseline serum creatinine values). Tables 1 and 2 show the demographic profile and clinical characteristics of the patients respectively. Maximum number of patients developed AKI (n=60; 57.14%) during the hospital stay. There was a dominance of stage I (n=39; 37.14%) over stage II (n=12; 11.42%) and stage III (n=12; 11.42%) in AKI patients (Tables 1 and 2).

On day 1, mean serum urea and serum creatinine levels were 68.71 ± 45.11 and 1.70 ± 0.91 mg/dl and on day 3, mean serum urea and serum creatinine levels were 106.02 ± 46.31 and 3.38 ± 1.80 mg/dl respectively. Value of mean ± SD of Computed Tomography Severity Score (CTSS), ESR, CRP, serum ferritin, serum procalcitonin level was 17.35 ± 2.81, 863.19 ± 346.30, 26.2 ± 9.47, 84.45 ± 50.65, 878.54 ± 544.44, 9.31 ± 10.87 respectively. The serum NGAL concentration (ng/ml) was significantly higher in patients who developed AKI compared to those who did not develop AKI (162.84 ± 91.70 vs. 42.88 ± 34.4, P=<0.001). Urinary NGAL level was similarly raised in patients with AKI (128.71 ± 71.60 ng/ml) than in patients without AKI (36.18 ± 37.38 ng/ml) (P=<0.001) (Table 3).

Table 3 depicts the various treatment outcomes and their comparison between the patients who did and did not develop AKI. A-Out of 60 patients who developed AKI, 15 patients (25%) required dialysis in the study. Development of AKI did not correlate with mortality in our study. Receiver operator curves were drawn to find out the cut-off value and the sensitivity, specificity of values of NGAL for prediction of AKI, as shown in Figure 1. AUC (Area Under Curve) for serum and urinary NGAL was 0.902, 0.857 respectively. Serum NGAL at values >91 was found to have a sensitivity of 85%, and specificity of 86.7% while urinary NGAL>80.6 has a sensitivity of 75% and specificity of 85% (p value<0.001) (Figure 1).

Univariate analysis showed the association of NGAL with need of dialysis. Hence, a multivariate analysis was done using a predictive model where serum NGAL, urinary NGAL, serum creatinine was considered as independent predictors of need of Dialysis. Serum and urinary NGAL showed significant predictive values with p value of 0.007 and 0.003 respectively although serum creatinine does not show significant value as shown in Table 4.

Correlation coeffecients were calculated between NGAL and CT severity scoring on HRCT Thorax. Spearman's coefficient of rank correlation was used for this purpose. Both serum and urinary NGAL were significantly related to CTSS scoring. Serum and urinary NGAL had correlation coefficient of 0.462 and 0.380 with p value 0.005 and 0.02 respectively when analysed with CTSS, as shown in Figures 2 and 3.

ROC curves were also drawn to find out the cut-off value and the sensitivity, specificity of values of NGAL for prediction of mortality in patients of severe COVID-19. AUC for Serum and urinary NGAL was 0.723, 0.699 respectively as shown in Figure 4. Serum NGAL at values >136.5 was found to have a sensitivity of 66.65%, and specificity of 76.7% while urinary NGAL>131 has a sensitivity of 58.33% and specificity of 75% (p-value<0.001) (Figures 4).

Table 1. Baseline demographic characteristics (n=105)

S. No	Characteristic	AKI (n=60)	Non AKI (n=45)
1	Mean Age ± SD (Range) in years	64.6 ± 16.42 (32-94)	50.87 ± 16.91 (10-70)
2	Sex- Male	33 (55%)	24 (53.3%)
	Female	27 (45%)	21 (46.67%)
3	Hypertension	39 (65%)	21 (46.67%)
4	Type 2 DM	36 (60%)	21 (46.67%)

Table 2. Association of different laboratory and radiological parameters with AKI (n=105).

S. No	Characteristic	AKI (n=60)		Non AKI (n=45)		Statistical significance	
		Mean	SD	Mean	SD	't'	'p'
1	Hb	11.155	2.71	11.08	2.08	-0.089	0.92
2	TLC	15405	6751.02	11080	4269.36	-2.172	0.03
3	Neutrophil	84.42	6.23	73.78	14.69	-2.919	0.006
4	Lymphocyte	9.99	5.08	21.03	14.81	3.111	0.003
5	NLR (neutrophil-lymphocyte ratio)	10.37	4.45	6.34	5.26	-2.453	0.01
6	ESR	26.2	9.47	20.13	8.29	-1.977	0.05
7	S. Bilirubin	0.93	0.62	0.66	0.33	-1.528	0.13
8	SGPT	81.69	68.49	76.53	57.85	-0.235	0.81
9	Serum urea (Day 1)	68.71	45.11	42.16	12.04	-2.214	0.03

10	Serum creatinine (Day 1)	1.7	0.91	1.21	0.29	-2.004	0.05
11	Serum urea (Day 3)	106.02	46.31	44.43	19.52	-4.825	<0.001
12	Serum creatinine (Day 3)	3.38	1.8	1.23	0.33	-4.553	<0.001
13	FBS	185.39	109.91	133.39	71.45	-1.594	0.12
14	HbA1C	7.47	2.75	6.75	1.6	-0.904	0.37
15	CRP	84.45	50.65	49.37	31.91	-2.351	0.02
16	Serum ferritin	878.54	544.44	928.79	679.72	0.243	0.8
17	D dimer	2.753	1.874	1.461	1.076	-2.386	0.02
18	Serum procalcitonin	9.31	10.87	4.02	4.52	-1.768	0.08
19	Serum NGAL	162.84	91.7	42.88	34.4	-4.805	<0.001
20	Urinary NGAL	128.71	71.6	36.18	37.38	-4.55	<0.001
21	Computed tomography severity Score-CTSS	17.35	2.81	15	3.56	-2.184	0.03

Table 3. Treatment outcomes with AKI and their correlation (n=105).

S. No	Characteristic	AKI (n=60)		Non AKI (n=45)		Statistical significance
1	Dialysis need	15 (25%)		0		$\chi 2=4.250;\ \pi=0.03$
2	Mech. Vent. Need	45 (75%)		21(46.6%)		$\chi 2=2.876;\ \pi=0.08$
3	Hospital stay (days)	8.1	3.97	6.73	4.44	$\chi 2=-0.961$ p=0.34
4	Mortality	27 (45%)		9 (20%)		$\chi 2=2.310;\ \pi=0.12$

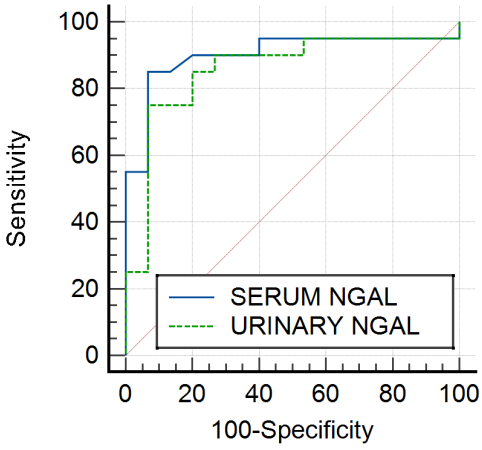

Figure 1. ROC Analysis for projection of cut-off values of S. NGAL and Urinary NGAL for prediction of AKI. The graph demonstrates the prediction of AKI using serum and urinary NGAL with a 95%confidence interval of 0.753 to 0.976 (p value<0.001) for serum NGAL and 0.697 to 0.952 (p value<0.001) for urinary NGAL respectively.

Table 4. Multivariant logistic regression analysis showing need of hemodialysis.

Variable	Coefficient	Std. Error	Wald	95% CI	P value
Serum NGAL	-0.0058637	0.019695	0.08864	1.01 to 1.20	0.007a
Urinary NGAL	0.022869	0.024946	0.8404	1.05 to 1.47	0.003b
Serum creatinine (Day 1)	1.08873	0.58004	3.5231	0.9530 to 9.2591	0.06
Note: NGAL:Neutrophil Gelatinase Associated Lipocalin					

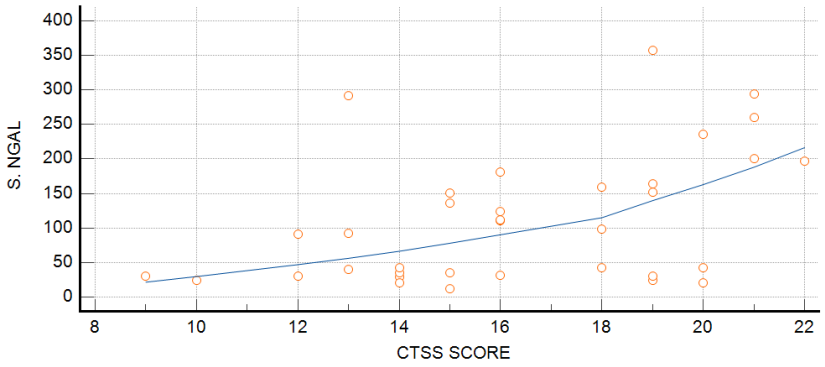

Figure 2. Scatterplot diagram showing correlation between Serum NGAL and CTSS score.

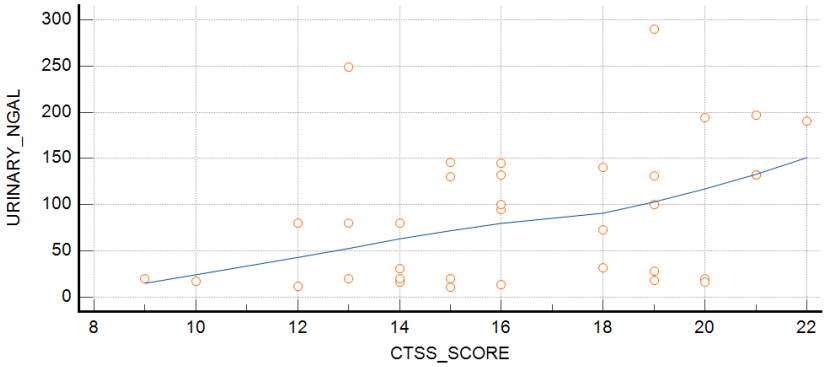

Figure 3. Scatterplot diagram showing correlation between Urinary NGAL and CTSS score.

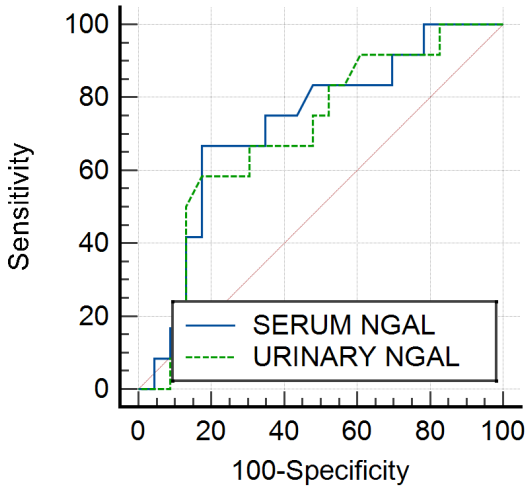

Figure 4. ROC Analysis curves for projection of cut-off values of S. NGAL and Urinary NGAL for predicting mortality. The graph demonstrates the prediction of mortality using serum and urinary NGAL with a 95%confidence interval of 0.546 to 0.860 (p value<0.001) for serum and 0.521 to 0.842 (p value<0.001) for urinary NGAL respectively.

Discussion

Renal injury in COVID 19 is due to various mechanisms such as cytopathic effect due to the virus itself, hypoperfusion, sepsis, cytokine storm induced systemic inflammatory response, complement activation, thrombosis and even due to imbalance of Renin Angiotensin Aldosterone System (RAAS) [11]. Epidemiology of COVID-19 associated AKI and its risk factors are still largely unknown and hence, this study is an attempt to bridge that gap. Finding an early predictor of AKI is of utmost importance as it can lead to its prevention, thus, decreasing morbidity and mortality.

In our study, 57.14% patients developed AKI which was similar to a study done by Aparicio et al. [12]. This was in contrast with some studies reporting low incidences of AKI between 7% in hospitalized patients with COVID-19 [13]. Differences between studies might be explained by the fact that all the institutions were referral centers for COVID-19 diseases, where most patients require ICU admission and only patients of severe COVID-19 disease were included.

We demonstrated that NGAL represents an independent risk factor to predict AKI in patients with COVID-19, suggesting a crucial role of NGAL in the diagnosis and prediction of AKI in COVID-19 patients.

In addition, NGAL also proved to be a strong predictor of all-cause mortality in this study. There could be two possible reasons for this finding. First, high levels of NGAL indicate an abrupt loss of kidney function resulting in AKI, which is significantly correlated with increased mortality [14]. Second, NGAL may be involved in the inflammatory processes and it might be responsible for the lethal complications of SARS-CoV-2 infection [15]. It has been reported that NGAL is an acute-phase protein likely to be elevated in many human diseases, particularly in the setting of inflammation, infection, and ischemia [14].

This is a pioneer study to evaluate the role of NGAL for AKI in COVID-19. He et al. [16] did a study on 174 patients and concluded that a high u-NGAL level, an increased GGO volume, and lymphopenia are strong predictors of a poor prognosis and a high risk of in-hospital death, which is also consistent with our results.

Our study also predicted the need of hemodialysis in COVID-19 patients using NGAL as a biomarker. This was consistent with the previous studies in critically ill patients. However, no such study has been done in patients of severe COVID-19 disease.

In our study, we also found an association between NGAL and CT severity score. On searching the literature, this study was not done earlier. This could also contribute to mortality and establishes the role of NGAL as an inflammmatory marker.

Conclusion

Both serum and urinary NGAL correlate strongly with development of AKI in patients of severe COVID-19. A single measurement of NGAL at the time of admission is an independent predictor of mortality. NGAL can also be used to determine the need of hemodialysis in patients of AKI in severe COVID-19 disease. It also strongly correlates with CT severity score and the lung changes in patients of severe COVID-19 disease. Thus, NGAL can be conveniently used as a prognostic marker in patients of severe COVID-19 disease.

Limitations and Recommendations for Future Studies

This study is a relatively new study done in COVID-19 patients. However, the study has a few limitations as well. The sample size was relatively low. The study though a multicentre one, did not pertain to different locations. Future researches on larger sample sizes are recommended. The study should also be done in other diseases involving respiratory tract, such as ARDS.

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