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Plasma NGAL (Neutrophil Gelatinase Associated Lipocalin) Levels in Septic Patients With & Without Acute Kidney Injury: A Plausible Biomarker

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ABSTRACT

Introduction: Acute kidney injury is extremely common among septic patients. Its early recognition among critically ill patients can help in better prognosis and decrease the risk of longer hospital stay and dialysis requirement. Creatinine is considered as a gold standard for diagnosis of Acute Kidney Injury but it has its own disadvantages. New biomarkers for Acute Kidney Injury that can help in its early recognition are the need of time. Many plasma and urinary markers including Neutrophil Gelatinase Associated Lipocalin are being studied. Its role in earlier detection of septic Acute Kidney Injury in septic patients in our set of population still needs extensive research where incidence of sepsis associated complications leading to death is extremely high.

Aims and Objectives: To measure plasma Neutrophil Gelatinase Associated Lipocalin levels in septic patients with and without acute injury compare pNGAL levels in two groups, and to measure specificity and sensitivity of plasma Neutrophil Gelatinase Associated Lipocalin levels in both sets of patients.

Place and Duration of study: A cross sectional analytical study was carried out in National Health Research Complex, Shaikh Zayed Hospital, Lahore over a period of 3 years from 2016-2019

Material and Methods: This is a cross-sectional analytical study carried out in ninety two patients that are divided in two groups on the basis of creatinine levels measured at 72hrs. First group consists of septic patients who developed Acute Kidney Injury (AKI) and second group consists of septic patients Without Acute Kidney Injury(WAKI). Plasma NGAL levels are compared in two groups. SPSS v 20 was used for analysis, p-value ≤ 0.05 was considered significant.

Results: The plasma NGAL level at 24 hours for group without Acute Kidney Injury was 126.4±15.4 ng/ml and for the group which developed Acute kidney Injury was 191.1±15.9 ng/ml and the difference was clearly significant with p-value <0.001.

Conclusion: pNGAL levels are significantly raised than the cut-off value of 150ng/ml at 0hrs in septic patients who developed AKI as compared to septic patients who didn't develop AKI.

Key Words: Sepsis, Acute kidney Injury, Plasma NGAL

INTRODUCTION

Acute Kidney Injury is a sudden fall in the kidney function and its foremost cause is sepsis[1].AKI is independently associated with complications, poor prognosis and high mortality[2]. Septic AKI is considered to be a distinct clinical entity. Sepsis is undoubtedly the most prevalent source of AKI today. Different analyses indicate that the incidence of septic AKI occurs between 19 to 48%, while the mortality of patients with septic AKI varies from 22 to 70%[3]. Tubular cellular injury is the most important in the contribution to sepsis induced AKI and the main molecular driver of injury is cellular hypoxia[4]. AKI intensity is almost proportional to the graveness of the inflammatory events involved in sepsis and the outcome. Septic AKI also has worse prognosis than non-septic AKI leading to higher mortality[5]. Creatinine and urine output have both been used to diagnose and determine the severity of septic AKI[6]. Specific symptoms that can help in early detection of AKI are lacking. In addition to it, creatinine is an insensitive and late marker for AKI.[7] Creatinine is affected by race, age, gender, muscle mass, nutrition status, hydration status, medications, catabolic rate and tubular secretion[8]. Serum creatinine has non-linear relationship with GFR and is not considerable as ideal marker for septic AKI as is troponin I considered for the diagnosis of acute myocardial infarction in the adult population[9].

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NGAL is a rapidly emerging marker for acute kidney injury. It is a small 25kD a protein covalently bound polypeptide from human neutrophils[10]. Neutrophil gelatinase associated lipocalin appears in two pools: systemic and renal. In healthy individuals, NGAL is continuously produced in various cell types and is also detectable in small amounts in systemic circulation[11]. In kidneys, it originates from distal convoluted tubule. It is expressed both in urine and plasma. It is freely filtered in urine through glomerulus[12]. It is readily reabsorbed in proximal tubule. So, normally minute amounts are detectable in urine[10]. Incase of AKI, NGAL is upregulated in ascending limb of loop of Henle, distal tubule and collecting duct leading to secretion from apical and basolateral part of nephron epithelia[13]. Re-absorption from proximal tubule is also impaired in case of proximal tubular injury which causes further increase in urine NGAL levels[14]. In septic patient, there are multiple sources of NGAL including extra renal production and release by neutrophils or epithelial cells into the bloodstream followed by glomerular filtration and then excretion in urine as well as increased production by renal tubular epithelial cell and by granulocytes sequestrated in tubular lumen[15]. Neutrophil gelatinase associated lipocalin can be measured in both blood and urine as a biomarker of AKI[16]. Role of pNGAL in septic patients has not been highlighted as required. Specially in Pakistan where the load of sepsis and AKI is much higher and the health care system is still deficient, the earlier diagnosis of septic AKI can definitely reduce the mortality rate and the financial resources requires during prolonged hospital stays.

MATERIAL AND METHODS

A cross sectional analytical study was carried out in National Health Research Complex, Shaikh Zayed Hospital, Lahore over a period of 2 years after approval from the Technical Review Board and the Ethical Committee. (IRB No: 1296). The sample size of eighty patients with expected occurrence of acute kidney injury as 60%. However, taking into account high mortality among septic patients, ninety-two patients with suspected or proven sepsis above the age of 18 years were included and patients with chronic renal insufficiency (sCr>3.0 mg/dl), on dialysis, pregnancy, urothelial, colorectal and pancreatic carcinomas were excluded. An informed consent was taken from each participant in the study. Demographic data was collected by a proforma. Detailed history and examination were carried out. Sepsis was defined as a systemic inflammatory response due to suspected or proven infection and was considered in the presence of WBC >12x103/mm3 at presentation and peripheral temperature >38°C persisting for at least 12 hours after presentation. AKI was defined as a 50% increase in serum creatinine levels from the baseline. A 3cc blood sample was collected at 0 hour for complete blood counts and creatinine levels. After normal creatinine levels were received, another 3cc blood sample was drawn for NGAL measurement within 24 hours and transported within an hour for centrifugation to separate plasma. pNGAL estimation was done by Lipocalin 2 (NGAL) Human ELISA kit. pNGAL levels of 150 ng/ml was considered as cut off value. Creatinine levels at 72 hours were again taken to determine whether the patient developed AKI .

Fifty-two patients developed AKI. However, 5 patients died and among the remaining forty-seven patients, forty patients were selected for septic AKI group after gender equalization with septic WAKI group to avoid any bias. Forty patients who developed acute kidney injury were assigned the group as septic AKI group and forty patients who didn't develop AKI were assigned the group as septic WAKI group.

SPSS version 20 was used for data entry and analysis. Serum creatinine at 0 hours and 72 hours was described by using Mean+ SD as well as median, Q1 and Q3 for both groups. Comparisons of creatinine at 0 hours between the groups was made by using Mann-Whitney U test and comparisons of creatinine at 72 hours between two groups was made by independent sample t-test. Data for plasma NGAL levels was described by using frequency and percentages for both groups and was also shown with box graphs. Comparison between the groups was made by independent sample t-test. Sensitivity of pNGAL for AKI at 24 hours was estimated by using ROC curve and was presented by using percentages with 95% confidence interval. p-value $\leq$ 0.05 was considered significant.

RESULTS

The study comprised ninety-two septic patients divided into two groups; acute kidney injury and without acute kidney injury. The mean±SD of the ages were 44.93±16.60 years for septic AKI and 47.45±15.95 years for septic WAKI. There were 23 males (57.5%) and 17 females (42.5%) in both acute kidney injury and without acute kidney injury group with Male to female ratio of 1.1:1. Regarding plasma NGAL at 24 hours, 40 patients (100%) had 151-226ng/ml plasma NGAL at 24 hours in AKI

and Non-AKI group, 40 patients (100%) had 96-150ng/ml plasma NGAL levels with mean and standard deviations 191.08±15.88 and 126.43±15.35 respectively. (Table-1) shows Frequency and percentage of plasma NGAL at 24 hours in both groups, (Table-2) shows Serum creatinine levels for two groups at baseline and at 72hours and comparison at each time, (Table-3) shows Comparison of plasma NGAL level at 24 hours between two groups, (Table-4) Acute kidney injury status by cut-off of plasma NGAL determined by ROC curve. While the Box plot presenting distribution of plasma NGAL for two groups is shown as Fig-1, the Receiver operative characteristics curve to determine cut-off for serum plasma NGAL level for acute kidney injury is elucidated as Fig-2.

Plasma NGAL at 24 hours	Acute kidney injury (n=40)		Without acute kidney injury (n=40)	
	No. of patients	% of patients	No. of patients	% of patients
96 – 150ng/ml	-	-	40	100.0
151 – 226ng/ml	40	100.0	-	-
Mean ±SD	191.08±15.88ng/ml		126.43±15.35ng/ml	
P-value	.000 (significant)			

Table-1: Frequency and percentage of plasma NGAL at 24 hours in both groups

Creatinine level at		Group of the patients: Acute kidney injury mg/dl	Group of the patients: Without AKI mg/dl	Test value	P-value
0 hour	Mean	1.2	1.0	Mann Whitney U = 620.5	0.080 (insignificant)
	SD	0.3	0.2		
	Q1	0.9	0.9		
	Median	1.1	1.0		
	Q3	1.4	1.2		
72 hour	Mean	2.7	1.3	t-test= 23.04	<0.001(significant)
	SD	0.3	0.2		
	Q1	2.5	1.2		
	Median	2.7	1.4		
	Q3	2.9	1.5		

Table-2: Serum creatinine levels for two groups at baseline and at 72hours and comparison at each time.

-	Group of the patients: Acute kidney injury ng/ml	Group of the patients: Without AKI ng/ml	t-test	P-value
Mean	191.1	126.4	18.51	<0.001 (significant)
SD	15.9	15.4		
Q1	180.0	114.5		
Median	190.0	129.5		
Q3	203.0	140.0		

Table-3: Comparison of plasma NGAL level at 24 hours between two groups.

Plasma NGAL at 24 hours	Group of the patients: Acute kidney injury N	%	Without AKI N	%	Total N	%
>150ng/ml	40	100.0	0	0.0	40	50.0
≤ 150ng/ml	0	0.0	40	100.0	40	50.0
Total	40	100.0	40	100.0	80	100.0

Table-4: Acute kidney injury status by cut-off of plasma NGAL determined by ROC curve

The ROC also confirmed non-overlapping with a cut-off 150 ng/ml, for all cases with acute kidney injury. The accuracy for predicting injury at 72 hours, through NGAL at 24 hours, was found 100% at this cut-off.

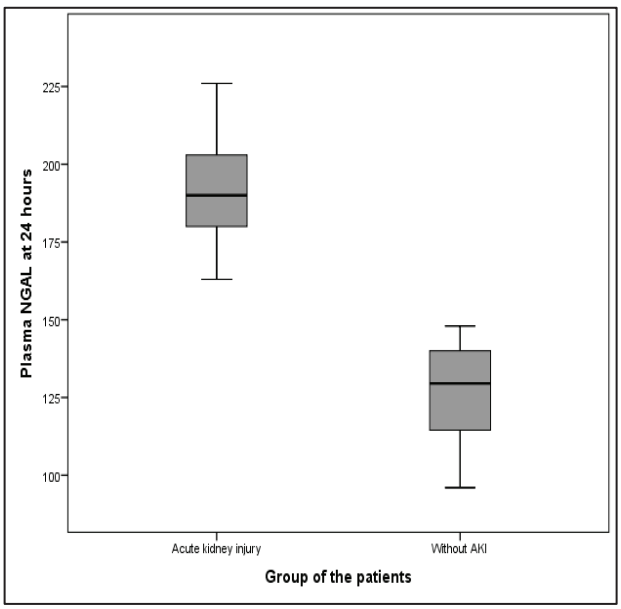

Fig-1: Box plot presenting distribution of plasma NGAL for two groups

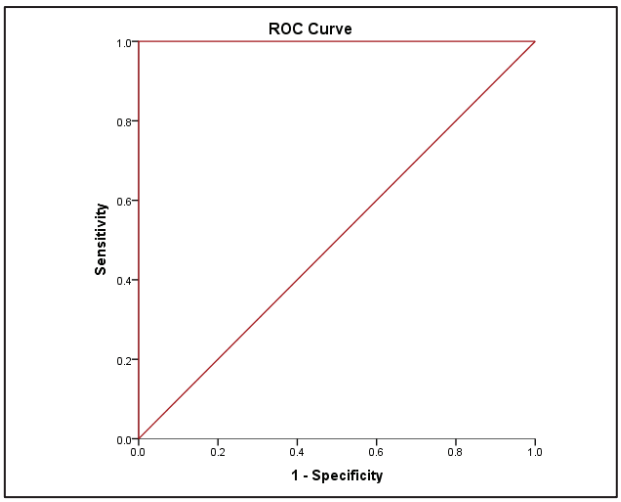

Fig-2: Receiver operative characteristics curve to determine cut-off for serum plasma NGAL level for acute kidney injury

DISCUSSION

Septic AKI takes a toll on overall health system resources and serum creatinine levels used solely as septic AKI marker have significant limitations[17]. In this study, it is analysed whether pNGAL holds its role as a biomarker in septic AKI in our population. Two groups each containing forty septic patients are made on the basis of AKI called septic AKI and septic WAKI and their creatinine levels and plasma NGAL levels are compared.

A cut of 150 nanogram per milliliter for plasma NGAL is considered[18]. For septic patients in different studies, a value of p NGAL within a range of 150 to 400 ng/ml has been considered[19]. The identification of AKI defined as a 50% or greater increase in serum creatinine levels in humans, is first described by Mishra et al and has been used as an AKI diagnostic criteria in a study carried out by Nisha et al[20]. In the study carried out by Shapiro et al, a total of 661 patients with suspected sepsis were finally analyzed for acute kidney injury. AKI is considered if serum creatinine level arises greater than 0.5mg/dl or acute need for RRT within 72 hours. The median NGAL levels for septic without AKI group carried out by Shapiro et al were 134 ng/ml and for septic AKI plasma NGAL median is 456 ng/ml. Plasma NGAL level in the group with AKI were 3.4 times higher than the plasma NGAL level in non AKI group. pNGAL levels greater than 150 ng/ml have area under curve of 0.82 thus showing that pNGAL is a strong predictor of septic AKI.[21]So, the results of this study validated the present study. However, the median pNGAL levels are much greater for septic AKI as well as without AKI as compared to those found in this study. Most probable reason being that in the study conducted by Shapiro et al [21], the patients included already had a higher creatinine level. In the current study the patients enrolled had normal creatinine levels however their pNGAL levels were still found to be proportionally raised to the level of acute kidney injury. This pointed towards the sensitivity of pNGAL as a biomarker for AKI matching the disease progression.

In a study carried out by Martensson J et all, the impact of sepsis on the concentration of NGAL in plasma and urine in adult ICU patients is analysed along with other biomarkers. The cut-off value for pNGAL is considered 120ng/ml. The median pNGAL value for Systemic inflammatory response syndrome (SIRS) patients without AKI is 111ng/ml, that for severe sepsis patients without AKI is 116ng/ml, and that for septic shock patients without AKI is 134ng/ml. The median pNGAL value for patients with septic shock with AKI is 216ng/ml. So, the difference between the pNGAL value of septic groups with AKI and without AKI is significant[22]. The results of this study are also comparable with those of this study showing the role of pNGAL as a potential biomarker for septic AKI patients. In the study carried out by Ralib et al, 129 septic patients were taken out of which 67 developed AKI. NGAL concentrations were higher in AKI patients within both the sepsis and SIRS patients (both $P < 0.0001$). AUC for septic AKI was 0.81 (95%CI: 0.74 to 0.87)[23]. In the study carried out by Khwaja et al, 48 patients with suspected sepsis were enrolled and plasma NGAL levels were measured at 12 hours and 24 hours and were found to be significantly higher in patients who developed AKI with AUC at 12 hours was 0.82 (95% CI 0.68-0.96) with a sensitivity of 70.8% and specificity of 90.9% thus further validating my study results[19].

This study has some limitations too. Only 80 patients were included. A greater sample size would be better. The time delay in the diagnosis of sepsis should be avoided using a better clinical approach. Temporal changes in the pNGAL should be considered by taking samples at 24 hours and 48 hours. So, further studies are recommended to establish NGAL as a biomarker for septic acute kidney injury patients.

CONCLUSION

This research has analysed the potential role of pNGAL as a more sensitive marker of septic AKI than creatinine. It has been statistically concluded that the plasma NGAL levels in septic patients that developed AKI are significantly higher than those who didn't develop AKI. It is a cost effective test as

it provides highest value for money when compared with the costs of the interventions after AKI has occurred like Renal Replacement Therapy with hemodialysis and peritoneal dialysis. Plasma NGAL levels at a cut-off value of 150ng/ml have shown high sensitivity (100%) and an AUC equal to 1, hence proving it to be a more authentic and dependable marker of Acute Kidney Injury in Septic patients and deleterious effects of AKI in septic patient can thus be prevented.

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