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RESEARCH ARTICLE

CLINICAL UTILITY OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (UNGAL) AS A MARKER IN DISCRIMINATION OF SEPTIC VERSUS NON-SEPTIC ACUTE KIDNEY INJURY

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Abstract

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Background & Aim: This study was aimed to determine whether there are unique patterns to urine neutrophil gelatinase-associated lipocalin (uNGAL) in septic compared with non-septic acute kidney injury (AKI)and also to evaluate whether uNGAL could discriminate between septic and non-septic AKI.

Materials& Methods: This prospective observational study screened critically ill AKI patientsand evaluated them for presenting complaints, and on evaluation, those who satisfied Systemic inflammatory response syndrome (SIRS) criteria were taken up for study. Those patients with evidence of infection and positive for at least two criteria for SIRS are taken up as cases (n=30) and the rest as controls (n=30). All the patients were serially monitored for urine output, renal parameters, urine NGAL, APACHE II score and SOFA score. Patient urine was collected immediately after admission, followed by 12h, 24 h & 48 h for measuring uNGAL.

Results: Mean change in uNGAL(ng/ml) at 12h, 24h and 48h was 12.5±5, 20.5±6.7 and 29.3±10.2 respectively in septic AKI and was 0.9±6.1, 0.9±6.3 and 0.1±6.1 respectively in non-septic AKI. Within the group, the analysis showed statistically high significance (p-value<0.0001) in septic AKI but not in the non-septic AKI group (p-value=0.97). On evaluating between-group analyses, we found a significantly high difference of NGAL in septic AKI at 12h, 24h and 48h compared to non-septic AKI(p-value<0.0001).

Conclusions: Septic AKI patients have higher detectable uNGAL than non-septic AKI patients. These differences in uNGAL values in septic AKI may have diagnostic and clinical relevance and pathogenetic implications.

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Introduction:-

Sepsis is a leading cause of acute kidney injury (AKI), accounting for up to 50% of cases in critically ill patients [1-6].Septic AKI portends a poorer prognosis with lower survival when compared with non-septic AKI [2,4,7,8].Septic AKI with early intervention may be associated with higher rates of renal recovery [3]. Considering these differences, the early identification of septic from non-septic AKI may improve the prognosis of the disease, which facilitates appropriate early treatment.

Septic AKI may be characterized by diverse pathophysiology that varies from ischemic/toxic-induced kidney injury [9–15]. This may be reflected in unique patterns of plasma and urine biomarkers in septic AKI [16,17].Consequently, the application of traditional urinary biochemical and microscopy-based tests in the early diagnosis and differentiation of AKI may be misleading in septic AKI [16-18]. Neutrophil gelatinase-associated lipocalin (NGAL) has shown potential as a valuable diagnostic biomarker in cases of AKI [19-21].Hence, this study was undertakento assess whetheruNGALcould discriminate between septic and non-septic AKI.

Materialsandmethods:-

This prospective, observational study was conducted at Narayana Medical College and Hospital, Nellore. This study was approved (Approval no.12/40/11)by the Institutional Ethics Committee, and written informed consent was obtained.Acute kidney injury (AKI) patients (defined by AKIN criteria) aged >18 years and admitted to the intensive care unit (ICU)with suspected sepsis with ananticipated in-hospital stay of more than 48h were considered forthe study. Patients were evaluated for presenting complaints & on evaluation, those who satisfied Systemic inflammatory response syndrome (SIRS) criteria were taken up for the study. Patients with evidence of infection and positive for at least two criteria for SIRS are taken up as cases (n=30) and the rest as controls (n=30).

Patients aged <18 years, prior h/o kidney disease like end-stage kidney disease (K/DOQI Stage V), renal replacement therapy (RRT) before ICU admission, or confirmed and/or suspected acute glomerulonephritis, interstitial nephritis, renal vasculitis or obstructive aetiology for AKI, Prior kidney transplant, diabetes mellitus, h/o malignancy, h/o receiving radiocontrast and/or nephrotoxic drugs 48-72h prior to admission were excluded.

All the study patients were serially monitored for urine output, renal parameters, urine NGAL, APACHE II score and SOFA score.

Study procedures:

All enrolled patients had indwelling urinary catheters. Patient's urine sampleswere drawn immediatelyafter 12h, 24h and 48h ofadmission for NGAL estimation. Biochemical parameters were analyzed immediately after admission (renal function test, serum electrolytes, arterial blood gas, cultures), and at 12h, 24h & 48h, renal function test, arterial blood gas, cultures, and serum electrolytes were repeated.

Estimation of uNGAL:

Urine samples collected for NGAL testing were immediately processed for supernatant (centrifugation for 10 mins at 1500 rpm). Overnight, samples are stored at -20°C after processing for batched analysis. Biochemical analysis was done by Biovendor human lipocalin-2/NGAL-Sandwich ELISA. Urine NGAL was expressed as ng/ml.

Statistical analysis:

Statistical analysis was performed using SPSS v20.0 (IBM Corp, Armonk, NY, USA). Categorical data was presented as actual numbers with percentages. For normally distributed data, independent student's 't-test and one-way repeated-measures analysis of variance (ANOVA) was used. Non-normally distributed data was analyzed by using the non-parametric Mann-Whitney U test. Fischer's exact and Chi-square tests were used to test the statistical significance of categorical variables. All the efficacy parameters will be presented as absolute changes from baseline. A negative sign indicates a decrease, and vice versa. For statistical significance, a two-tailed p-value of <0.05 will be considered.

Results:-

In this study, 30 patients with septic AKI and 30 with non-septic AKI were taken as cases and controls, respectively. Male: female ratio in septic AKI and non-septic AKI was 22:08 and 20:10 respectively (p-value=0.57). Patients mean age was 55.2±12.3 vs 58.3±10.2 yrs in cases and control group respectively, (p-value=0.29). We found significantly higher APACHE II score in cases as compared to controls (24.2±6.2 vs. 18.7±8.1 respectively, p-value=0.004). Similarly, the SOFA score was statistically significant in cases as compared to the control group (8.6±2.6 vs 6.6±2.8, p-value=0.006). Patients with septic AKI had higher mechanical ventilation use than non-septic AKI group (p=0.27) (Table1).

Table 1:- Baseline Characteristics.

Variable	Septic AKI (n=30)	Non-Septic AKI (n=30)	p-value
Gender (Male:Female)	22:08	20:10	0.57
Age (years)	58.3±10.2	55.2±12.3	0.29
APACHE II Score	24.2±6.2	18.7±8.1	0.004*
SOFA II Score	8.6±2.6	6.6±2.8	0.006*
Mechanical Ventilation	12 (40.0%)	08 (26.7%)	0.27

AKI: acute kidney injury. *Indicates significant p-value.

On evaluation of physiological parameters at baseline, we found significantly high heart rate (110±18 vs. 86±15 beats/min; p-value<0.0001), respiratory rate (24±4 vs. 16±5 breath/min; p-value<0.0001), temperature (101±0.5 vs. 98±0.6 F; p-value<0.0001), WBC count (13000±1200 vs 9500±960 mm^3/ml; p-value<0.0001) in septic AKI group as compared to non-septic AKI group. However, there was significantly low mean arterial pressure (62.4±12.6 vs 68.3±13.6 mmHg; p-value=0.05), Pao2/Fio2 ratio (216±90 vs. 284±84; p-value=0.003), Urine output (65±8 vs. 70±10; p-value=0.04) in septic AKI group as compared to non-septic AKI group. All the patients in the case group and 23 patients in the control group had SIRS scores of more than two and were statistically significant(p-value=0.01), as shown in Table2.

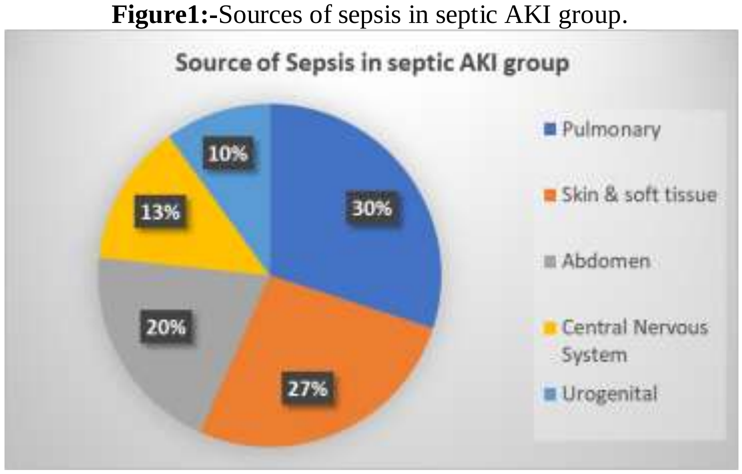
Table2:-Baseline Physiological Characteristics.

Variable	Septic AKI (n=30)	Non-Septic AKI (n=30)	p-value
Heart rate (bpm)	110±18	86±15	<0.0001*
Respiratory rate (breaths/min)	24±4	16±5	<0.0001*
Temperature (F)	101±0.5	98±0.6	<0.0001*
Mean arterial pressure (mmHg)	61.4±12.6	68.3±13.6	0.04*
>2 SIRS (%)	30 (100%)	23 (76.7%)	0.01*
Pao2/Fio2 ratio	216±90	284±84	0.003*
WBC count (mm^3/ml)	13000±1200	9500±960	<0.0001*
Urine output (ml/hr)	65±8	70±10	0.04*

AKI: acute kidney injury, bpm: beats per minute, SIRS: Systemic inflammatory response syndrome, WBC: white blood cells.

The source of sepsis in the Septic AKI group was most commonly pulmonary (30.0%) followed by skin and soft tissue (26.7%), abdomen (20%), CNS (13.3%) and urogenital (10%), as shown in figure1.

Figure1:-Sources of sepsis in septic AKI group.



Assessment of baseline kidney function showed no significant difference in serum creatinine (1.5±0.6 vs. 1.3±0.5 mg/dl; p-value=0.17) andserum urea (60±12 vs. 52±10 mg/dl; p-value=0.26) levels between septic AKI and non-septic AKI groups. Similarly, on analyzing kidney function after enrollment, we did not find a statistically significant difference in serum creatinine (1.8±0.5 vs. 1.6±0.4 mg/dl;p-value=0.09) andserum urea (96±10 vs. 92±13 mg/dl; p-value=0.18) levels between septic and non-septic AKI groups.

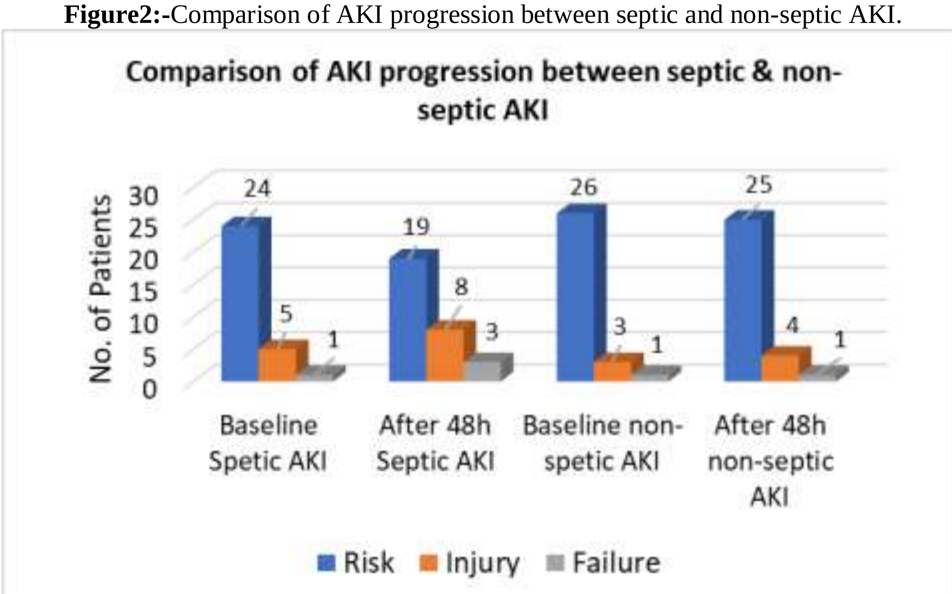
As shown in Table 3 and Figure2, among the septic AKI group, 80.0% of patients were classified as risk, 16.7% as injured and 3.3% of patients failure as per AKIN criteria at baseline. After 48h, 63.3% were in risk category, 26.7% patients in injury and 10.0% patients were considered as failure. After 48h, within Septic AKI, there was no significant change in AKIN criteria. Similarly, among non-septic AKI, 86.0% of patients were considered as risk, 10.0% of patients as injury and the remaining 3.3% as failure at baseline. After 48h, 83.3% were in risk category, 13.3% patients in injury and 3.3% patients were considered as failure. There was no significant change in AKIN criteria within non-septic AKI after 48 hrs. Between the groups, no significant difference was seen (p-value=0.81).

Table3:-Comparison of AKI progression as per AKIN criteria.

AKIN Criteria	**Septic AKI**			**Non-septic AKI**		
	Baseline	**After 48h**	**p-value**	**Baseline**	**After 48h**	**p-value**
Risk	24 (80.0%)	19 (63.3%)	0.17	26 (86.7%)	25 (83.3%)	0.78
Injury	05 (16.7%)	08 (26.7%)		03 (10.0%)	04 (13.3%)	
Failure	01 (3.3%)	03 (10.0%)		01 (3.3%)	01 (3.3%)	
	Between the groups p=0.81					

AKI: acute kidney injury.

Figure2:-Comparison of AKI progression between septic and non-septic AKI.



Mean change in NGAL (ng/ml) at 12h, 24h and 48h was -12.5±5, -20.5±6.7 and -29.3±10.2 respectively in septic AKI group and was -0.9±6.1, -0.9±6.3 and -0.1±6.1 respectively in non-septic AKI group. Within-group analysis showed a statistically significant change in the septic AKI group (p-value<0.0001) but not in the non-septic AKI group (p-value=0.97). Inter-group analysis showed a significantly high difference (p-value<0.0001)in septic AKI as compared to the non-septic AKI group at 12h, 24h and 48h (Table4).

Table4:-Comparison of mean change in urinary NGAL.

	Mean change in uNGAL (ng/ml) from baseline			**p-value**
	at 12h	**at 24h**	**at 48h**	
Septic AKI	-12.5±5.0	-20.5±6.7	-29.3±10.2	<0.0001*
Non-septic AKI	-0.9±6.1	-0.9±6.3	-0.1±6.1	0.97

p-value between groups	<0.0001*	<0.0001*	<0.0001*	

*Indicates significant p-value. AKI: acute kidney injury; uNGAL: urinary neutrophil gelatinase associated lipocalin.

Discussion:-

Acute kidney injury (AKI) is a frequent and severe complication in ICU patients with sepsis [22], particularly in the elderly [23]. It is strongly evidenced that sepsis and septic shock are the primary causes of acute kidney injury (AKI) in critically ill patients. They account for over 50% of AKI cases in ICUs and are linked with a very high mortality rate [1].

Serum creatinine is an unreliable and delayed marker of kidney dysfunction, which is generally used in the diagnosis of AKI. Serum creatinine level rises when there is a significant deterioration in the renal function. Physiological levels may be modified by age, gender, ethnicity, dietary protein intake, muscle mass or metabolism, hydration status, and drugs, and can remain within the reference range despite severe renal impairment in low muscle mass patients [24,25].

Serum creatinine, blood urea nitrogen (BUN) or urine markers of kidney injury do not directly reflect cell injury but relatively the delayed functional consequences of the damage. Identifying early stages of AKI is challenging due to the lack of reliable biomarkers that are sensitive and specific. NGAL has recently emerged as a novel biomarker for AKI resulting from various etiologies, including cardiac surgery, contrast nephropathy, kidney transplantation, and sepsis.

Serum and urine NGAL levels increase about 48 hours prior to creatinine rise and strongly correlate with changes in creatinine concentrations[26]. ELISA can measure urine levels of NGAL and is a very sensitive marker of acute kidney injury, which can increase up to 1000-fold under certain conditions like post-cardiac surgery [27].Early diagnosis of AKI enables prompt therapeutic intervention and improves outcomes.

Our study findings showed that septic AKI was associated with higher initial values of uNGAL, which persisted for the duration of 48h when compared with non-septic AKI. We found an association ofseptic AKI with peak uNGALbut not with non-septic AKI. Also, higher uNGAL showed fair discrimination for a diagnosis of septic versus non-septic AKI. We also found significant differences across numerous clinical, physiologic and laboratory parameters between septic AKI and non-septic AKI.Despite similar AKI severity at enrollment, septic AKI patients were generally older and had high APACHE II and SOFA II score. These differences have likewise been described in prior studies [2,3,28].

We found that septic AKI was associated with higher levels of uNGAL. One hypothesis to potentially explain this observation is that sepsis induces a greater kidney injury than other contributing factors. While higher NGAL levels have been associated with greater severity of AKI, no investigation has examined differences in the expression of NGAL by contributing factors for AKI. Alternatively, NGAL may have higher expression in inflammatory states such as sepsis or in selected malignancies. Indeed, Wheeler et al. found higher values of pNGAL in children with septic shock compared to those with either SIRS or healthy controls [29]. NGAL is expressed in several tissues, including the lung, and up-regulated in cancer [30-32]. Likewise, increased NGAL expression is associated with acute inflammation and/or infection [30,31,33]. These observations suggest NGAL may represent an emerging global biomarker for inflammation, tissue injury, illness severity and organ failure and correlate with survival in sepsis.

However, the study by Nickolas et al. did not find the superiority of NGAL level over serum creatinine level in predicting subsequent AKI in patients admitted to an emergency department, given that patients already with AKI and those with subclinical AKI were included [21].From a future perspective, exosomal transcription factors may be used to identify kidney injury. [34].

Conclusion:-

Detectable urine NGAL levels are higher in septic AKI when compared with non-septic AKI, which may have diagnostic and clinical significance.

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Conflict of Interest:
None to declare.

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