The prognostic value of neutrophil gelatinase-associated lipocalin in sepsis-associated acute kidney injury: A prospective observational study

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ABSTRACT

Background: Sepsis is one of the most common triggering factors for acute kidney injury (AKI). The aim of the study is to evaluate the outcome in sepsis with AKI and determine the prognostic value of urinary neutrophil gelatinase-associated lipocalin (NGAL) in septicemic AKI.

Materials and Methods: This prospective follow-up study was carried out over a period of 1 year after ethical clearance from the Institutional Ethics committee, a total 165 cases of septicemia were recruited, of which 15 patients were dropped out, 150 patients were identified suffering from septicemia defined as per the organ dysfunction criteria (according to third international consensus 2016) and patients of AKI defined as per the Kidney Disease Improving Global Outcomes 2012 criteria).

Results: Out of 150 patients of septicemia enrolled in the study, only 38 (25.33%) suffering from AKI were classified as Group I and rest 112 (74.67%) patients of septicemia not suffering from AKI were classified as Group II. In total, 60.0% (90) patients were discharged from the hospital, rest of the patients (40%) expired. Mean duration of survival was higher in Group II (21.29 ± 1.89 days) as compared to Group I (13.67 ± 1.06 days). Cases with ≥121.90 urine NGAL, rate of mortality (41.7%), were higher as compared to alive patients discharged (34.4%).

Conclusion: Sequential organ failure assessment score, hospital stay, and mortality were high in septicemic patients with AKI as compared to sepsis without AKI. Survival of patients also not good with septic AKI, those patients who had high NGAL value had poor prognosis.

Key Words: Acute kidney injury, mortality, sepsis, sequential organ failure assessment score, survival

INTRODUCTION

Sepsis is one of the most common triggering factors for acute kidney injury (AKI) in critically ill patients. In a prospective study, sepsis was recognized as the most important contributing factor for AKI, and the rate is approximately 50%.¹ Other studies reported that 40%–75% of AKI were associated with sepsis.²⁻⁴ These patients generally had a poor prognosis when compared to septic non-AKI.⁵⁻⁷ Therefore, early detection of septi
AKI patients is of great importance to enable adequate treatment in these patients and improve their outcomes. AKI incidence rate and severity correlate with the severity of the underlying sepsis.[9]

Pathophysiology of Acute kidney injury is complex, it not only ischemic damage it varies according to etiological triggers.[10] Kidney injury might be a combination of immunologic, toxic, and inflammatory damage that may affect the microvasculature and the tubular cells.[9]

In the current clinical practice, AKI is diagnosed by measuring blood urea nitrogen (BUN) and serum creatinine, but it is well recognized that BUN and serum creatinine are insensitive and late indicators of AKI.[10] More recently, urine neutrophil gelatinase-associated lipocalin (uNGAL) has been considered more sensitive and specific test to detect AKI.[11] However, uNGAL is also a marker of systemic inflammation, since it is typically released by neutrophils upon activation.[12] Hence, it is extremely important to determine the relationship between uNGAL and septic AKI. The aim of this study is to evaluate the outcome in sepsis with AKI (in terms of sequential organ failure assessment [SOFA] score, morbidity, duration of hospital stay, and mortality) and determine the prognostic value of urinary NAGL in septicemic AKI.

**MATERIALS AND METHODS**

This prospective follow-up study was carried out over a period of 1 year in the Department of Internal Medicine, Nephrology Unit in collaboration with the Department of Pathology, King George’s Medical University, Lucknow. Ethical clearance was obtained from the Institutional Ethics Committee, Research Cell, King George’s Medical University, Lucknow, and after informed consent, a total of 165 cases of septicemia were recruited for the study, of which 15 patients were dropped out, 150 patients were identified suffering from septicemia as per the organ dysfunction criteria (according to the third international consensus 2016) and patients of AKI defined as per the Kidney Disease Improving Global Outcomes (KDIGO) 2012 criteria were enrolled for this study. Thus, 38 patients of septicemia with AKI and 112 patients of septicemia without AKI who fulfilling the inclusion criteria were enrolled in the study.

Patients with age group of >18 years, who were admitted in intensive care unit with evidence of sepsis, were included in this study. Patients who had end-stage renal disease, glomerular filtration rate <15 ml/min/m², acute glomerulonephritis and acute interstitial nephritis, renal vasculitis or postrenal etiology for AKI, or who had renal transplant and on chronic dialysis therapy were excluded from the study.

AKI defined as per the KDIGO 2012.[13]

Increase in serum creatinine by ≥0.3 mg/dl (≥26.5 mol/L) within 48 h or increase in serum creatinine to ≥1.5 times from baseline or urine volume 0.5 ml/kg/h for 6 h. AKI is staged as, Stage 1: increase in serum creatinine by 1.5–1.9 times from baseline; or increase in serum creatinine by ≥0.3 mg/dl (≥26.5 mol/L); or urine output 0.5 ml/kg/h for 6–12 h, Stage 2: increase in serum creatinine by 2.0–2.9 times baseline; or urine output ≤ 0.5 ml/kg/h for 12 h, and Stage 3: increase in serum creatinine by 3.0 times from baseline; or increase in serum creatinine to 4.0 mg/dl (353.6 mol/L); or initiation of renal replacement therapy.

According to the third international consensus 2016, sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[14] Clinical criteria are suspected or documented infection and an acute increase of ≥2 SOFA points.[14] About 5 ml venous blood samples were obtained within the first 24 h of intensive care unit (ICU) admission and then every next day for 3 days from all patients and centrifuged at 5000 rpm for 10 min at room temperature, serum was separated for routine hematology biochemistry test. Urine samples were collected on day 1, either from spontaneous voids or from indwelling Foley catheters for urinalysis, and urine protein measurements as per the study protocols.

Automated blood cell analyzer (Abbott CELL-DYN Ruby Hematology Analyzer) was used for routine hematology testing, and an automated clinical biochemistry analyzer (Elitech Selectra PROM) for BUN, creatinine, electrolytes, serum bilirubin, and albumin testing. Enzyme-linked immunosorbent assay (ELISA) technique was used to measure urine NGAL (uNGAL) levels. A clean, morning midstream urine sample (5 mL) was collected into a sterile test tube and centrifuged at 5000 rpm for 15 min. The supernatant was transferred to an Eppendorf tube and stored at ~80°C until uNGAL was measured. A human NGAL ELISA kit (Epitope Diagnostics, Inc., San Diego, CA, USA) was used for estimation of uNGAL as per the manufacturer’s protocol. Serum creatinine was measured by Jaffe’s method and its normal range was 0.8–1.2 mg/dl.

SPSS 15 version IBM (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL, USA). Results were analyzed as mean ± standard deviation and percentiles or median (range) values. The independent samples t-test, and Chi-square and Mann–Whitney U-tests were used for comparison of the categorical and continuous variables. NGAL values, sepsis and non-AKI, and sepsis and AKI groups were evaluated and compared with by one-way analysis of variance. P < 0.05 was considered statistically significant.
RESULTS

Out of 150 patients of septicemia enrolled in the study, only 38 (25.33%) suffering from AKI were classified as Group I and rest 112 (74.67%) patients of septicemia not suffering from AKI were classified as Group II. Out of 38 (25.33%) septic AKI patients, 28.95% (11) Stage I AKI, 34.21% (13) Stage 2 AKI, and 36.84% (14) Stage III AKI were found on the basis of serum creatinine level observed every 3 days of duration [Table 1].

Difference in SOFA score of patients of Group I (4.39 ± 1.13) and Group II (3.33 ± 0.86) was found to be statistically highly significant \( P < 0.001 \) [Table 2].

60.0% (90) patients were discharged from the hospital, rest of the patients (40%) expired, though higher number of patients of Group II (sepsis without AKI) (61.61%) were discharged in uneventful condition as compared to Group I (sepsis with AKI) (55.26%) and difference was statistically not significant [Figure 1].

Out of 150 patients, 40.0% (60) expired; mortality rate was higher in Group I (44.7%) as compared to Group II (38.4%). Mean duration of survival was higher in Group II (21.29 ± 1.89 days) as compared to Group I (13.67 ± 1.06 days), but this difference was statistically not significant [Table 3 and Figure 2].

Urinary NGAL level of patients of Group I (231.09 ± 64.03) was found to be significantly higher as compared to that of Group II (119.04 ± 32.57) [Table 4].

DISCUSSION

In the present study, patients age ranged from 18 to 85 years and majority of patients in both the groups were ≤40 years of age. Although the mean age of patients of Group I (37.97 ± 18.50 years) was higher as compared to Group II (33.43 ± 17.83 years), the difference was nonsignificant. Another study also reported higher mean age of septic AKI patients as compared to septic non-AKI patients \( (P = 0.200) \).[15]

The present study result showed that advance age was much prone for development of AKI. Another author reported the mean age of septic AKI was 70 ± 13 years.[16]

In the present study, Group I had high SOFA score (mean 4.39) and more prone to develop AKI as compared to Group II (mean SOFA 3.00), and this difference was statistically significant \( (P < 0.001) \). Another study reported a significantly higher SOFA score in the septic AKI patients as compared to non-AKI sepsis \( (P < 0.01) \).[17]

In our study, hospital stay was significantly prolonged in septic AKI patients as compared to sepsis without

| Table 1: Distribution of patients in two groups (septic acute kidney injury and septic nonacute kidney injury) and grade of acute kidney injury among septic acute kidney injury patients |
|---|
| **Group** | **Description** | **Number of patients (n = 150), n (%)** | **Grade of AKI** | **Number of patients (n = 38), n (%)** |
| Group I | Septic AKI | 38 (25.33) | Stage I | 11 (28.95) |
| | | | Stage II | 13 (34.21) |
| | | | Stage III | 14 (36.84) |
| Group II | Septic non-AKI | 112 (74.67) | | |

AKI: Acute kidney injury

\[Figure 1: \text{Comparison of outcomes among Group I and Group II. } \chi^2 = 0.476 \ (df = 1); \ P = 0.490 \]

\[Figure 2: \text{Survival analysis for septic acute kidney injury and nonacute kidney injury septic population} \]
AKI (mean value of 10.50 days in Group I vs. 7.64 days in Group II) \((P = 0.011)\). Similarly, mortality was high in septic AKI group as compared to sepsis without AKI (44.74% in AKI group vs. 38.39% in non-AKI group) \((P = 0.490)\). Similarly reported by another author where duration of hospital stay was higher in sepsis AKI group \((24 \pm 17)\) as compared to septic non-AKI group \((18 \pm 11)\). Similarly, mortality was high in septic AKI group \((67\%)\) as compared to septic non-AKI group \((24\%)\).[16]

In sepsis, the kidney is one of the most commonly affected organs almost 47.0% AKI cases are associated with sepsis.[1]

Previous studies showing increased concentration of NGAL level in sepsis regardless of the presence or absence of AKI.[15] This specific characteristics make it difficult to use NGAL to identify AKI associated with sepsis. While another study demonstrated that NGAL seems to be a useful predictor of AKI in patients with sepsis, although NGAL concentration did not differ significantly between AKI and non-AKI patients.[11] In our study, NGAL value was high in patients with septicemia-associated AKI as compared to septicemia without AKI. Day 1 urinary NGAL was good prognostic marker in septic patients with AKI. Similarly, another author also reported higher value of urinary NGAL in AKI with sepsis as compared to sepsis without AKI and they also found good predictive power of NGAL for identifying AKI with sepsis.[18]

Among cases with \(\geq 121.90 \) uNGAL, prognosis was poor; number of deceased was higher (41.7%) as compared to number of alive and discharge (34.4%).

The values of urine NGAL were measured from sample collected on day 1. Patient developing AKI (Group I) had significantly higher value of uNGAL \((231.09 \pm 64.03 \) vs. \(119.04 \pm 32.57\)) even on day 1 \((P < 0.001)\). One author suggested that plasma NGAL was raised in patients with systemic inflammatory response syndrome, severe sepsis, and septic shock and should be used with caution as a marker of AKI in ICU patients with septic shock, and suggested that uNGAL is more useful in predicting AKI, as the levels are not elevated in septic patients without AKI.[15]

### CONCLUSION

Outcome of the patients in terms of hospital stay and mortality was high in septicemic patients with AKI as compared to sepsis without AKI. Those patients who had high NGAL value had poor prognosis. Significant difference in mean value of NGAL was found between septic AKI and nonseptic AKI. Urinary NGAL concentration was associated with severity of disease and has good prognostic value.

| Table 2: Comparison of SOFA score among Group I and Group II |
|---------------------------------|
| **Group** | **n** | **Score minimum** | **Score maximum** | **Median** | **Mean ± SD** | **P** |
| Group I | 38 | 3 | 7 | 4.00 | 4.39 ± 1.13 | 0.001* |
| Group II | 112 | 2 | 5 | 3.00 | 3.33 ± 0.86 | 0.003 |
| **Total** | 150 | 2 | 7 | 4.00 | 3.60 ± 1.04 | 0.003 |

\(Z = 4.873\) (Mann–Whitney U-test); \(*P < 0.001\) (significant). SD: Standard deviation, SOFA: Sequential organ failure assessment

| Table 3: Survival analysis for septic acute kidney injury and nonacute kidney injury septic population |
|---------------------------------|
| **Group** | **Total** | **Number of mortalities (%)** | **Mean survival time ± SE (days)** | **P** |
| Group I (septic AKI) | 38 | 17 (44.7) | 13.67 ± 1.06 | 0.356 |
| Group II (septic non-AKI) | 112 | 43 (38.4) | 21.29 ± 1.89 | 0.001* |
| **Overall** | 150 | 60 (40.0) | 18.68 ± 1.70 | 0.001 |

Log-rank (Mantel–Cox) \(x^2 = 0.850; P = 0.356\) (NS). AKI: Acute kidney injury, SE: Standard error, NS: Not significant

| Table 4: Urine neutrophil gelatinase-associated lipocalin levels among Group I and Group II and association of grade of acute kidney injury with neutrophil gelatinase-associated lipocalin among septic acute kidney injury patients |
|---------------------------------|
| **Group** | **n** | **Range** | **Mean ± SD** | **P** | **Grade** | **NGAL** |
| | | | | | | **Range** | **Mean ± SD** |
| Group I | 38 | 116.80-356.20 | 231.09 ± 64.03 | 0.001* | Stage I \((n = 11)\) | 146.70-308.40 | 231.05 ± 57.65 |
| | | | | | Stage II \((n = 13)\) | 116.80-314.80 | 232.12 ± 71.16 |
| | | | | | Stage III \((n = 14)\) | 148.60-356.20 | 230.16 ± 66.60 |
| | | | | | \(F, P\) | | 0.003; 0.997 |
| Group II | 112 | 64.30-221.60 | 119.04 ± 32.57 | | | |
| Total | 150 | 64.30-356.20 | 147.43 ± 64.80 | | | |

\(t = 13.988; *P < 0.001\) (significant). SD: Standard deviation, NGAL: Neutrophil gelatinase-associated lipocalin

| Table 5: Association of outcome with neutrophil gelatinase-associated lipocalin |
|---------------------------------|
| **Number of alive patients** \((n = 90), n (%)\) | **Number of expired patients** \((n = 60), n (%)\) | **Statistical significance** |
| **NGAL** | | **P** |
| At cut off of NGAL value \((\geq 121.90)\) | 31 (34.4) | 25 (41.7) | 0.807 | 0.370 |

NGAL: Neutrophil gelatinase-associated lipocalin
Research quality and ethics statement
The authors of this manuscript declare that this scientific work complies with reporting quality, formatting, and reproducibility guidelines set forth by the EQUATOR Network. The authors also attest that this clinical investigation was determined to require Institutional Ethics Committee, Research Cell, King George’s Medical University, Lucknow and appropriate approval (84th ECM II-B-Thesis) was granted by the Research Cell, King George’s Medical University, Lucknow.

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Conflicts of interest
There are no conflicts of interest.

Ethical conduct of research
This study was approved by the Institutional Review Board / Ethics Committee. The authors followed applicable EQUATOR Network (http://www.equator-network.org/) guidelines during the conduct of this research project.

REFERENCES

1. Singbartl K, Kellum JA. AKI in the ICU: Definition, epidemiology, risk stratification, and outcomes. Kidney Int 2012;81:819-25.
2. Neveu H, Kleinknecht D, Brivet F, Loirat P, Landais P. Prognostic factors in acute renal failure due to sepsis. Results of a prospective multicentre study. The French study group on acute renal failure. Nephrol Dial Transplant 1996;11:293-9.
3. Silvester W, Bellomo R, Cole L. Epidemiology, management, and outcome of severe acute renal failure of critical illness in Australia. Crit Care Med 2001;29:1910-5.
4. Bagshaw SM, Laupland KB, Doig CJ, Mortis G, Fick GH, Mucenski M, et al. Prognosis for long-term survival and renal recovery in critically ill patients with severe acute renal failure: A population-based study. Crit Care 2005;9:R700-9.
5. Bagshaw SM, Uchino S, Bellomo R, Morimatsu H, Morgera S, Schetz M, et al. Septic acute kidney injury in critically ill patients: Clinical characteristics and outcomes. Clin J Am Soc Nephrol 2007;2:431-9.
6. Oppert M, Engel C, Brunkhorst FM, Bogatsch H, Reinhart K, Frei U, et al. Acute renal failure in patients with severe sepsis and septic shock: A significant independent risk factor for mortality: Results from the German prevalence study. Nephrol Dial Transplant 2008;23:904-9.
7. Bagshaw SM, George C, Bellomo R. ANZICS Database Management Committee. Early acute kidney injury and sepsis: A multicentre evaluation. Crit Care 2008;12:R47.
8. Ishikawa K, May CN, Gobe G, Langenberg C, Bellomo R. Pathophysiology of septic acute kidney injury: A different view of tubular injury. Contrib Nephrol 2010;165:18-27.
9. Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: What do we really know? Crit Care Med 2008;36:S198-203.
10. Abraham BP, Frazier EA, Morrow WR, Blasak RT, Devarajan P, Mittnäfes M, et al. Cystatin C and neutrophil gelatinase-associated lipocalin as markers of renal function in pediatric heart transplant recipients. Pediatr Transplant 2011;15:564-9.
11. Kim H, Hur M, Cruz DN, Moon HW, Yun YM. Plasma neutrophil gelatinase-associated lipocalin as a biomarker for acute kidney injury in critically ill patients with suspected sepsis. Clin Biochem 2013;46:1414-8.
12. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovitch N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008;36:1297-303.
13. Acute Kidney Injury Work Group. Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for acute kidney injury. Kidney Int 2012;2:1-138.
14. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 2016;315:801-10.
15. Mårtensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. Intensive Care Med 2010;36:1333-40.
16. Aydoğdu M, Gürsel G, Sancak B, Yenı S, Sari G, Taşyürek S, et al. The use of plasma and urine neutrophil gelatinase associated lipocalin (NGAL) and cystatin C in early diagnosis of septic acute kidney injury in critically ill patients. Dis Markers 2013;34:237-46.
17. Dai X, Zeng Z, Fu C, Zhang S, Cai Y, Chen Z. Diagnostic value of neutrophil gelatinase-associated lipocalin, cystatin C, and soluble triggering receptor expressed on myeloid cells-1 in critically ill patients with sepsis-associated acute kidney injury. Crit Care 2015;19:223.
18. Park HS, Kim JW, Lee KR, Hong DY, Park SO, Kim SY, et al. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury in sepsis patients in the emergency department. Clin Chim Acta 2019;495:552-5.