Original Article JCMCTA 2022 ; 33 (1) : 173-177

Diagnostic Value of Urinary Neutrophil Gelatinase-Associated Lipocalin in Detecting Acute Kidney Injury in Asphyxiated Term Neonates

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Abstract

Background: Neonates with Perinatal Asphyxia (PNA) are at higher risk for developing Acute Kidney Injury (AKI). Conventionally, Serum Creatinine (SCr) is used to diagnose AKI but lacks specificityduring the first few days of life. Neutrophil Gelatinase-Associated Lipocalin (NGAL) has emerged as a novel biomarker of AKI in such situations, while its value in our setting is yet to be validated. The study aimed to evaluate the role of urinary NGAL (uNGAL) level as a predictive biomarker of AKI in term newborn infants with PNA.

Materials and methods: This prospective observational study included 55 full-term neonates with PNA from the Special Care Neonatal Unit of Chittagong Medical College Hospital.The uNGAL was measured by an enzyme-linked immunosorbent assay. Urinary samples were obtained for uNGAL on postnatal day 1(after 6 hours), and on day 3, blood samples for sCr were obtained on postnatal day1, 3 and 6.

Results: Among 55 neonates with PNA, 20 (36.4%) had AKI. The mean ±SD uNGAL level was 97.77±17.13 ng/ml in neonates with AKI compared to 25.76±2.19 ng/ml in those without AKI in the first postnatal day. An uNGAL cutoff value of 28.86 ng/ml had 90.0% sensitivity and 82.9% specificity in predicting AKI the same day. The mean value of uNGAL was observed at a lower level,54.53±14.72 ng/ml vs 15.17±2.18 ng/ml in AKI and no AKI neonates on third postnatal days. The cutoff value for detecting AKI was seen to be 26.43 ng/ml with high validity on this day. Significant differences in sCr were observed between AKI and no AKI measuring on days one and three, whereas the difference was not significant on day six.

Conclusion: The study showed that uNGAL might be a valuable biomarker for predicting AKI among neonates with PNA.

Key words: Acute kidney injury; Neutrophil gelatinase-associated lipocalin; Perinatal asphyxia.

Introduction

Perinatal Asphyxia (PNA) remains a common problem in Neonatal Intensive Care Units (NICU). It is a significant cause of morbidity and death in term and preterm neonates, especially in low-resource settings.[1,2] PNA can lead to multiorgan dysfunction and redistribution of cardiac output to maintain cerebral, cardiac and adrenal perfusion while potentially compromising renal, gastrointestinal and skin perfusion.[3,4] So, Acute Kidney Injury (AKI) is common in the asphyxiated neonate and the incidences range between 30% to 56%.[3-7]Early detection of AKI could optimize and improve patient outcomes.[8] A major difficulty in diagnosing this condition is the lack of a consensus definition of neonatal AKI, largely because of a shortage of specific, measurable variables and biochemical markers. Commonly used renal parameters like Serum Creatinine (sCr) and urine output have major limitations.[9]

Identifying biomarkers improving AKI detection is mandatory to improve PNA management and improve the adult prognosis.[6] Functional genomic studies identified Neutrophil Gelatinase-Associated Lipocalin (NGAL) as the earliest induced protein after renal ischemic injury in rats.[7] NGAL is a 26-kDa protein of the lipocalin family constitutionally expressed by several tissues and neutrophils. Its expression is upregulated during inflammation, infection and

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Submitted on : 11.03.2022
Accepted on : 16.04.2022

ischemic events. NGAL is partially absorbed in the proximal tubule in normal conditions. In case of renal injury, the kidney produces NGAL, rising 6–12h after the insult, proportionally to the severity of the aggression.[8] Pediatric studies have shown that urinary NGAL (uNGAL) can predict, with a good performance, AKI and dialysis requirement in the postoperative pediatric cardiac surgery period.[9,10] Recently, serum and uNGAL represent candidate biomarkers with high performance in predicting AKI in newborns with PNA.[11]

However, information regarding the level of uNGAL, especially in Bangladeshi asphyxiated neonates, is scarce. Population-specific studies are needed to define the optimal cutoffs and determine which levels of uNGAL suggest post-asphyxial AKI.This study aimed to determine the predictive value of uNGAL in detecting AKI in asphyxiated neonates admitted to a tertiary hospital in Bangladesh.

Materials and methods

This prospective observational study was conducted in the Special Care Neonatal Unit (SCANU) of Chittagong Medical College Hospital, Chattogram, Bangladesh, for one year from April 2019 to March 2020. Before starting the study, ethical clearance was obtained from the Ethical Review Committee of Chittagong Medical College, and informed written consent was taken from the parents of the patients.

Fifty-five full-term (>37 weeks), both sexes, neonates had clinical and laboratory findings of PNA according to the American Academy of Pediatrics criteria and showing Hypoxic Ischemic Encephalopathy (HIE) Stage II and III were included in the study.[12] Neonates of a mother suffered from diabetes mellitus, pre-eclampsia, chronic kidney disease, or received nephrotoxic therapy, a newborn infant with sepsis, major congenital anomalies, an inborn error of metabolism, birth weight <2500g, preterm infants, neonates with birth trauma or antenatal USG showing fetal renal anomalies were excluded from the study.

All participants were subjected to the following: i) Full history taking, including gestational age, birth weight, sex, and mode of delivery. ii) Full clinical examination and recording of Apgar scores at 1 and 5 min. iii) Laboratory investigations: included uNGAL measurement on day 1, day 3, and sCr measurement on day 1, day 3, and day 6 of life. UNGAL estimation was done using the Argutus Medical Human NGAL ELISA kit (Invitrogen, made in Austria, the year 2020, batch BMS 2202, lott 211654007) in one batch at the end of sample collection. Estimation of sCr was carried out immediately using the Creatinine Humalyzer 3000 assay.

Patients were divided according to the level of sCr in two groups: AKI: level of sCr≥133 μmol/l (≥1.5mg/dl) or a percentage increase in sCr of ≥50 % (1.5-fold from the baseline) (n=20).[13] No-AKI: serum creatinine level ≥1.5mg dL (n=35).

Data were analyzed using (SPSS) Statistical Package of Social Science for windows software Version 23. Descriptive data were expressed as number (no.), percentage (%) mean, Standard Deviation (SD) and range. uNGAL levels were expressed as median and interquartile range due to the nonparametric nature of the data. The sensitivity and specificity for the cutoff level of uNGAL were calculated and plotted as a Receiver Operating Characteristic curve (ROC curve) for visual analysis and determination of the optimal cutoff value of uNGAL. Chi-Squared test was used to compare two or more qualitative variables, student's t-test was used to test the difference between two means of normally distributed data, and Mann-Whitney U test for nonparametric data. Values of $p < 0.05$ were considered significant.

Results

Of the enrolled asphyxiated neonates, 20 (36.4%) developed AKI. Asphyxiated neonates with AKI were comparable to neonates without AKI regarding demographic and clinical characteristics (Table I).

Table I Demographic and clinical prenatal characteristics of the studied neonates stratified by their AKI status (n=55)

Characteristics	No AKI (n=35)	AKI (n=20)	p value
Gestational age (Week)	38. 83(±1.36)	38.90 (±1.25)	0.842†
Mode of delivery			
NVD	24 (68.6)	16 (80.0)	0.144*
CS	11 (31.4)	4 (20.0)	
Sex of neonate			
Male	23 (65.7)	17 (85.0)	0.122*
Female	12 (34.3)	3 (15.0)	
Birth weight (Kg)	2.83 (±0.29)	2.93 (±0.37)	0.282†
APGAR score			
At 1 minute	4 (3-5)	3.5 (3-5)	0.881‡
At 5 minute	4 (3-5)	4 (3-5)	0.703‡
HIE stage			
Stage II	25 (71.4)	17 (85.0)	0.254*
Stage III	10 (28.6)	3 (15.0)	

NVD: Normal Vaginal Delivery, CS: Cesarean Section, HIE: Hypoxic-Ischemic Encephalopathy. †Independet sample t-test. *Chi-square test. ‡Mann-Whitney U test.

Mean (±SD) uNGAL levels on daysone and three were significantly higher in patients with AKI than those without AKI. There was a highly statistically significant difference in sCr uNGAL level in patients with AKI than those without AKI on days one andthree but not on day 6 (Table II). Correlation between uNGAL and sCr at day 1 and day 3 showed significant co-relation in the study (r=0.857, p<0.001, r=0.394, p=0.017).

Table II Comparison of serum creatinine and uNGAL between AKI and no AKI subgroups of the studied neonates (n=55)

Parameters	Day	Mean±SD values in neonates with No AKI	AKI	p value†
S. creatinine (mg/dl)	1[a]	0.93±0.21	1.80±0.15	<0.001
	3[b]	0.74±0.17	0.99±0.31	0.003
	6[c]	0.68±0.12	0.81±0.22	0.063
uNGAL (ng/ml)	1[a]	25.76±2.19	97.77±17.13	<0.001
	3[b]	15.17±2.18	54.53±14.72	<0.001

[a,b,c] Number of studied neonates in no AKI and AKI groups on D1, D3, and D6 were 35, 20; 21, 17; and 14, 15 respectively. †Independent sample t-tests.

ROC analysis of the data showed that the best cut off value of day one uNGAL was value 28.86 ng/mL, this value could predict AKI in asphyxiated neonates, with an area under the curve (AUC) of 0.851 and a confidence interval of (0.726- 0.977) < 0.001. uNGAL had a sensitivity of 90.0%, specificity 82.9%, positive predictive value 75.0% and negative predictive value 93.6% (Figure 1) (Table III). The second ROC curve shows that day three uNGAL can predict AKI at the cutoff point of (26.43 ng/mL) with a sensitivity of 87.5% and specificity of 90.5% (Figure 2) (Table III).

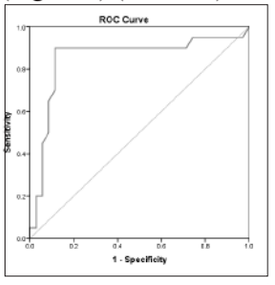

Figure 1 ROC curves of uNGAL in diagnosing AKI after perinatal asphyxia in Day 1 of lif

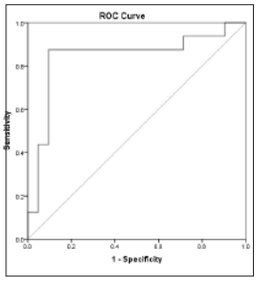

Figure 2 ROC curves of uNGAL in detecting AKI in asphyxiated neonates in 3rd postnatal day

Table III Validity and predictivity of uNGAL (Day 1 and 3) in detecting AKI in asphyxiated neonates

uNGAL cutoff level	Sen (%)	Spe (%)	PPV (%)	NPV (%)	AUC	95% CI of AUC	p value
Day 1							
28.86 ng/ml	90.0	82.9	75.0	93.6	0.851	0.726- 0.977	<0.001
Day 3							
26.43 ng/ml	87.5	90.5	87.5	90.5	0.842	0.693-0.991	<0.001

Sen: Sensitivity, Spe: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area Under the Curve, CI: Confidence Interval.

Discussion

Early recognition of AKI in asphyxiated newborns is necessary for decreasing morbidity and mortality. The present study has evaluated the association of uNGAL a urine biomarkers with AKI in asphyxiated neonates and found that uNGAL level in urine was associated with AKI. These data suggest the potential role of uNGAL as biomarkers of AKI in asphyxiated neonates.

The frequency of AKI in the present study was 36.4%. Previous studies have reported a broad range of incidence of AKI after PNA, with rates as high as 55% to as low as 18.5% in neonates with PNA.[14-18] The AKI incidence rate was comparable to the earlier study by Zhang et al and Oncel et al.[16,18] On the other hand, the rate of the present study was lesser than that observed by Tanigasalam et al and Abdelhady et al and higher than that observed by Tanzil et al and Essajee et al.[16,15,17,14] Other than the small sample sizes, the variation may be due to variable definition of AKI across studies as well as the variation of the severity of PNA in the studies.

In the present study, uNGAL obtained on the first day of life showed a significantly higher mean value in asphyxiated neonates with AKI than neonates without AKI. Though this was in agreement with other similar studies conducted in different countries, the uNGAL values differ significantly.[14-18] Further studies are essential to explain such variation in the uNGAL level among studies.

In the present study, ROC curve analysis suggested that the uNGAL cutoff value of 28.86 ng/ml on day 1 of life in neonates with PNA can predict AKI development with the sensitivity of 90%, specificity of 82.86%, PPV of 75.0%, and NPV of 93.55%. The used cutoff values of uNGAL level presented wide variation (18–652 ng/ml)

in the studies, rendering thus the interpretation of the test problematic.[9-18] The differences in these studies could be related to the number of patients enrolled, the prevalence of the disease, and whether the study was designed primarily for identifying NGAL levels to diagnose AKI. The exciting finding of the present study is its AUC value which was 0.851. Studies to date show AUC values ranging as high as 0.93 to as low as 0.724.[14,15]

An important finding in this study is how NGAL behaves as AKI progresses. The general trend of uNGAL shows similar levels at days 1 and 3, with a statistically significant difference between AKI and no AKI group. A comparison of levels of uNGAL on day 1 for patients with and without AKI shows statistically significantly higher levels of NGAL in the former than in the latter.The present study established that mean sCr at day one was 1.80 mg/dl vs 0.9 mg/dl in AKI and no AKI that was significant with $p<0.001$, and at day three, it decreased to 0.99 mg/dl vs 0.74 mg/dl in AKI and no AKI that was significant with $p<0.003$, but at day sixdifference was not significant. The present study established a significant positive correlation between uNGAL and sCr on day one and day 3 of life, both in the AKI subgroup and no AKI group. This was similar to Abdelhady et al.'s study in the AKI subgroup but not in the no AKI group.[15]

We set strict selection criteria to handle the confounders, excluding neonates with sepsis. None of the included neonates in this study had any sepsis risk factors. All newborns included in the study were term neonates. So there is less chance of gestational age as a confounding factor. Since gender was another important confounding variable, female and male neonates' distribution was analyzed in AKI and no AKI groups. It was found similar in the present study.

Effective early risk assessment is crucial to identify the group of asphyxiated neonates who would benefit from applying the above preventive strategies. uNGAL values obtained on day one of life can predict the AKI with 90% sensitivity and 82.86% specificity in the present study. This finding has important implications in management strategies and the counselling of parents.[6] In this context, uNGAL should be evaluated in conjunction with other novel biomarkers and clinical factors to construct a combined predictive model that would maximize the accuracy of AKI detection among newborns with PNA.

Limitations

The sample size was small and collected from a single centre. The lack of a matched control group of healthy neonates was another limitation in this work.

Conclusion

The present work reflects that uNGAL represents a promising bio-marker detecting AKI in asphyxiated neonates. In asphyxiated neonates, AKI can be evaluated early, even in the first postnatal day, by detecting uNGAL. Thus the tool may bring a favourable change in AKI management in the newborn with PNA through avoidance of nephrotoxic agents and early institution of appropriate treatment.

Recommendations

uNGAL might be used as a diagnostic tool in addition to sCr for early diagnosis of AKI for time management.Large-scale multi-centre prospective studies with random sampling might be conducted to accurately see the diagnostic efficacy of NGAL with its value in asphyxiated neonates with AKI. Moreover, the present study findings justify the validation of this AKI biomarker, especially to clinically important endpoints such as the need for dialysis, length of hospital stay or mortality in this condition.

Acknowledgement

Authors express gratitude to the parents of respondents.

Contribution of authors

MH-Conception, data collection, drafting & final approval.
SB-Data collection, drafting & final approval.
MSI-Data analysis, critical revision & final approval.
MU-Interpretation of data, drafting & final approval.
NS-Interpretation of data, critical revision & final approval.
FMAH-Data collection, drafting & final approval.
MJBAC-Data collection, drafting & final approval.
JD-Design, critical revision & final approval.

Disclosure

All the authors declared no competing interest.

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