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Original Research Article

URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AS AN EARLY PREDICTOR OF AKI IN ASPHYXIATED NEONATES

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ABSTRACT

Background: Acute kidney injury (AKI) is a complication of perinatal asphyxia seen in 46% of these neonates[1,2]. Serum creatinine is an unreliable measure in the acute setting[3]. In order to improve the prevention, early diagnosis and treatment of AKI in new born, an early biomarker of renal injury like Neutrophil Gelatinase-Associated Lipocalin NGAL is required.
Materials and Methods: Hospital based Prospective cohort study conducted in NICU, S.V.R.R.G.G.H, Tirupati. Study population are term neonates admitted on day 1 of life with birth asphyxia. Newborns with congenital anomalies, chromosomal abnormalities, suspected IEM, sepsis; babies born to mothers with diabetes, preeclampsia and multiple gestation, and those received nephrotoxic drugs were excluded. Sample size was 40 neonates. For the study participants, history was elicited and physical examination was done. Urine sample for NGAL was collected for all the 40 neonates within 24 hr of life. Serum creatinine and urine output were monitored for AKI. The data was analyzed with Epi info version 7.2.0 software.
Results: The prevalence of AKI was found to be 42.5% among term babies with birth asphyxia. ROC curve analysis suggested that a urine NGAL cut off value of 51.53 ng/ml, the test has a sensitivity of 88.2% and the specificity of 91.3%. ROC analysis shows area under the curve (AUC) is more than 0.9 and the p value of less than 0.001 indicates that the test has favourable sensitivity and specificity. 64.7% of asphyxiated neonates had oliguric AKI and 35.3% had non oliguric AKI.
Conclusion: High urine NGAL level is significantly associated with the subsequent diagnosis of AKI in asphyxiated neonates. So early measurement of this biomarker in moderate and severely asphyxiated neonates can reliably predict AKI due to birth asphyxia.
Key words: Acute Kidney Injury, Neutrophil Gelatinase Associated Lipocalin NGAL, Birth asphyxia, Serum Creatinine.

INTRODUCTION

Acute kidney injury (AKI) is a common consequence of perinatal asphyxia occurring in up to 46% of these infants.[1,2] Detection of reduced kidney function with a rise in serum creatinine concentration, is an unreliable measure in the acute setting,[3] and it is elevated in late stages when AKI is almost irreversible. So the establishment of non-serum creatinine-based AKI diagnostic criteria is crucial for this age group. In order to improve the prevention, diagnosis, treatment, prognosis and early prediction of AKI, novel early markers of AKI are required.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a 25kDa secretory glycoprotein that belongs to the lipocalin family of proteins. Renal expression of NGAL increases dramatically after renal ischemia. This is reflected by the rapid rise in urinary NGAL reported in AKI.[4] Several experimental and clinical studies have shown that the expression of urine and serum NGAL increases significantly in AKI. In particular, the urine NGAL level is closely associated with the severity of kidney injury, and could be detected earlier than other markers of AKI.[5] Therefore, NGAL shows potential to be a new effective early biochemical marker of AKI.

Aim and Objective: To asses NGAL as an early predictor of AKI in asphyxiated term neonates.

MATERIALS AND METHODS

A Hospital based Prospective Cohort Study conducted during June 2023 to June 2024 in NICU, Dept of Pediatrics, S.V.R.R.G.G.H attached to S.V. Medical College, Tirupati. Study subjects are term neonates with birth weight appropriate for gestational age admitted on day 1 of life with moderate and severe birth asphyxia according to NNF criteria. Before collection of data, one or both parents were briefed about the purpose of the study and a written informed consent was obtained. All the tests and procedures were done free of cost and no financial burden was imposed on the parents. Newborn with congenital malformations, chromosomal abnormalities, suspected inborn error of metabolism, sepsis; those born to diabetic or preeclamptic mothers; outcomes of multiple gestations, and those born to mothers who received nephrotoxic drugs were excluded from the study. A total sample of 40 neonates with moderate and severe birth asphyxia were included in the study.

Methodology: All term AGA neonates admitted on Day 1 of life with moderate and severe birth asphyxia were screened for inclusion and exclusion criteria. After inclusion of newborns with birth asphyxia, a complete history was elicited and physical examination was done with special emphasis on neurological examination. Urine sample for NGAL was collected for all the 40 neonates within 24 hrs of life and sent to regional MRU for storage and analysis. Laboratory investigations including complete blood count, serum CRP were sent. Serum creatinine and urine output were monitored for development of AKI. AKI was defined based on AKIN (Acute Kidney Injury Network) criteria for serum creatinine. Neonates with > 0.3 mg/dl rise in serum creatinine from the baseline, a percentage increase in serum creatinine of ≥ 50% (1.5-fold from baseline) were diagnosed to have AKI.[5] Oliguria was defined as urine output < 1 mL /kg/ hour. Babies were monitored till day 7 of life.

Statistical analysis: Data was entered into window Excel and analyzed by descriptive statistics. The results were reported as number (percentage) for categorical variables, mean ± standard deviation (SD) for normally distributed variables. ROC curve was analyzed using Epi Info version 7.2.0 software.

Ethical Considerations

1. Before the collection of data all the parents were briefed about the procedure and purpose of the study and then written informed consent was obtained from the parent of the subject.
2. All investigations related to the study were done free of cost and no financial burden was imposed on parents of the subject.
3. Confidentiality of individual information was maintained.
4. No conflict of interest.

RESULTS

A total of 40 neonates with moderate and severe birth asphyxia based on NNF criteria were recruited in the study among which 25 were male babies and 15 were female babies. Out of 40 babies with moderate and severe birth asphyxia, 17 babies (42.5%) had AKI and 23 babies (57.5%) did not have AKI.

Table 1: Baseline characteristics of the study participants

Parameter	Values
Sex	
Male n(%)	25 (62.5%)
Female n(%)	15 (37.5%)
Birth weight in grams (mean)	2915+/-159.5
Perinatal Asphyxia	
Moderate (%)	26 (65)
Severe (%)	14 (35)
HIE	
Stage I (%)	15 (37.5)
Stage II(%)	18 (45)
Stage III(%)	7 (17.5)
Oliguria n(%)	21(52.5)
Non oliguria n(%)	19 (47.5)
Urinary NGAL ng/ml (mean+/-SD)	47 +/- 24.5

Moderate asphyxia	42.7+/- 19.3
Severe Asphyxia	55.7+/- 30
In AKI	66 +/- 21
In non AKI	33+/-16
AKI n(%)	17 (42.5)
No AKI n(%)	23 (57.5)
OUTCOME	
Death n(%)	13 (32.5)
Discharge n(%)	27 (67.5)

Table 2: Association of severity of birth asphyxia and oliguria in AKI

	Clinical parameter	**AKI present**	**AKI absent**	**Total**
1	**Birth Asphyxia**			
	Moderate asphyxia	11 (40.7%)	16 (59.3%)	27 (100%)
	Severe asphyxia	6 (46.2%)	7 (53.8%)	13 (100%)
2	**Urine output**			
	Oliguria	11 (64.7%)	10 (43.5%)	21
	Non oliguria	6 (35.3%)	13 (56.5%)	19
	Total	17	23	40

Out of 27 babies with moderate birth asphyxia, 11 babies (40.7%) had AKI. Among 13 babies with severe asphyxia 6 babies (46.2%) had AKI. The association between severity of birth asphyxia and AKI is statistically not significant (p value 0.74) as the p value is > 0.05. Among 17 babies with AKI, 11 babies had oliguria and 6 babies had normal or increased urine output suggesting that 64.7% of babies with AKI had oliguric AKI and 35.3% had non oliguric AKI. The association of oliguria with AKI is statistically not significant (p value 0.18) as the p value is > 0.05. Out of 17 babies with AKI, 5 babies (29.4%) died and 12 babies (70.6%) were discharged. Mean urine NGAL values in babies with AKI and without AKI are 66 +/- 21 ng/ml and 33+/- 16 ng/ml respectively.

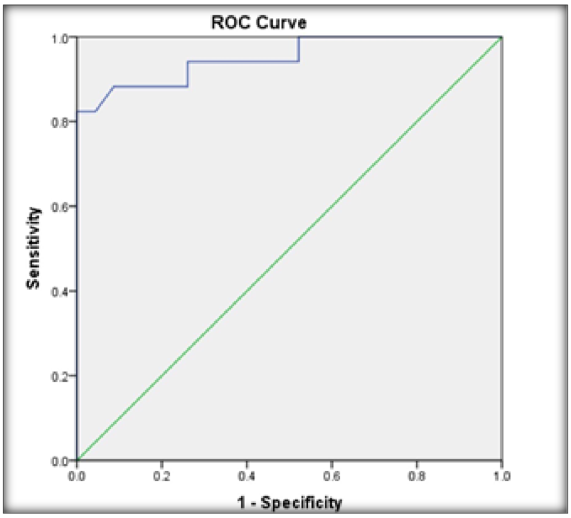

Figure 1: ROC curve for urine NGAL

Table 3:

Area Under the Curve				
Test Result Variable(s): urinary NGAL Day 1				
Area	Std. Error[a].	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.95	0.035	0	0.881	1

ROC Analysis shows area under the ROC curve (AUC) was more than 0.9, with Confidence Interval of 0.881-1.00 the p value of less than 0.001 indicates that the test has favorable sensitivity and specificity.

Table 4: Diagnostic ability of urine NGAL with respect to AKI in asphyxiated neonates

Sensitivity	88.4 %
Specificity	91.3%
Cut off value	51.53 ng/ml

With a cut off of 51.53 ng/ml, urinary NGAL test sensitivity of detecting AKI was increased to 88.2% and the chance of detecting false positives is 8.7 % ie., specificity of 91.3%.

Among the newborn with moderate and severe birth asphyxia, 42.8% of babies with HIE-3, 44% babies with HIE 2 and 40% babies with HIE-1 have urine NGAL > 51.53 ng/ml.

Table 5: Correlation between Urinary NGAL with other variables in the study participants with AKI (n=17)

Variable	**U NGAL<51.53 ng/ml**	**U NGAL<51.53 ng/ml**	**Total (n=17)**
Gender			
Male n (%)	1 (8.3)	11(91.7)	12

Female n (%)	1 (20)	4 (80)	5
Oliguria			
Present n (%)	2 (18.1)	9 (81.9)	11
Absent n (%)	0 (0)	6 (100)	6
Perinatal Asphyxia			
Moderate n(%)	2 (18.1)	9 (81.9)	11
Severe n (%)	0 (0)	6 (100)	6
HIE			
Stage 1 n(%)	1(14.2)	6 (75.8)	7
Stage 2 n(%)	1 (14.2)	6 (75.8)	7
Stage 3 n(%)	0	3 (100)	3
Outcome			
Death n(%)	0	5 (100)	5
Discharge n (%)	2 (17)	10 (83)	12

DISCUSSION

Birth asphyxia is one of the leading causes of mortality and morbidity in newborn. AKI is independently associated with poor outcome in the critically ill newborn. The standard biomarker of kidney function, serum creatinine, shows a noticeable increase in concentration many hours to days after renal insult. Thus, creatinine based AKI diagnosis is likely to be delayed, rendering therapy to mitigate or prevent AKI ineffective. Further creatinine concentration reflects the maternal level for up to 72 hours after birth, making it unhelpful in the assessment of the neonate in the initial 2-3 days of after birth. Hence biomarkers are gaining importance. Moreover, there is significant variability in neonatal creatinine levels and GFR, because of changes in neonatal physiology as the baby adapts to extrauterine life in the immediate postnatal period. Many studies show the high prevalence of AKI in asphyxiated newborns, as 29.5% by Bozkurt and Yucesoy,[7] 54% by Gupta et al,[8] and 72% by Hankins et al.[9] In the present study the prevalence of AKI was found to be 42.5% among term babies with moderate and severe birth asphyxia.

NGAL levels increase significantly in the presence of inflammation and injury of epithelia secondary to ischemic or nephrotoxic insult. Consequently, NGAL significantly rises in blood and urine soon after AKI.[10] In this study, urine NGAL levels were significantly higher in cases with acute kidney injury than cases without AKI. This was in agreement with another study done on asphyxiated neonates which found that asphyxiated neonates had significantly higher urine NGAL.[11] Similarly, the study done by Krawczeski, et al.[3] observed that both plasma and urine NGAL concentrations became markedly and significantly higher in both neonatal and non neonatal patients with AKI.

The current study shows that 64.7% of asphyxiated neonates had oliguric AKI and 35.3% had non oliguric AKI. Similarly oliguria has been reported to be higher among asphyxiated neonates by other authors with figures ranging from 25% to 69.2% babies.[12,13] In a study by Raggal NE et al., they observed that only 20% of AKI cases had oliguria while 80% had normal urine output[14]. Non-oliguric renal failure is a recognized entity secondary to perinatal asphyxia. Renal parenchymal injury in non oliguric as well as oliguric renal failure is essentially similar but heterogenous response of individual nephron and variable damage to tubular epithelium results in oliguric AKI in some neonates and non oliguric AKI in other babies. If there is less severe damage to tubular epithelium, it causes decrease in fractional reabsorption which exceeds the decrease in single nephron GFR leading to normal or increased urine output in non-oliguric renal failure.[15]

In the study done by Sarafidis et al., on asphyxiated neonates, they found that asphyxiated neonates had significantly higher serum and urinary NGAL values than the controls at all-time points,[16] which is similar to the present study. Similarly, the study of Sweetman DU & Molloy EJ done on the biomarkers of acute kidney injury in neonatal encephalopathy, they observed that asphyxiated neonates had significantly elevated urinary NGAL and they concluded that asphyxiated neonates did suffer acute tubular injury.[17]

In our study, ROC curve analysis suggested that a urine NGAL cut off value of 51.53 ng/mL within the first 24 hours of life in asphyxiated neonates can predict the development of AKI with sensitivity of 88.2% and the specificity of 91.3 %. Receiver Operating Characteristic (ROC) analysis shows area under the curve (AUC) was more than 0.9 and the p value of less than 0.001 indicates that the test has favourable sensitivity and specificity. The results of the present study are comparable to the results of the study done by Sarafidis et al., which showed that urine NGAL at cut off values of > 18.61 ng/mL had 100 % sensitivity, 83.3 % specificity, to detect Acute Kidney Injury (AKI) in asphyxiated neonates.[16] In a study on AKI in asphyxiated neonates conducted by Zhung Y et al, they demonstrated test sensitivity of 84.6% and specificity of 88% for a urine NGAL cut off of 109.5 µg/L.[5]

The present study is limited by the relatively small sample size and done in term neonates only. Further large scale studies are needed to study about the association of AKI with respect to gender, gestational age and birth weight including premature and low birth weight neonates are needed.

CONCLUSION

In the present study it is concluded that urine NGAL level is early and specific biomarker of AKI in term neonates with birth asphyxia. High urine NGAL level was significantly associated with the subsequent diagnosis of AKI in these neonates. So early measurement of this biomarker in moderate and severely asphyxiated neonates can reliably predict the development of AKI due to ischemic insult of birth asphyxia.

Conflict of Interest: Nil

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