RESEARCH PAPER

Serum Neutrophil Gelatinase-Associated Lipocalin as a Marker of Acute Kidney Injury in Asphyxiated Neonates

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Objective: To determine the clinical utility of serum neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in asphyxiated neonates with hypoxic ischemic encephalopathy (HIE).

Design: Cohort study.

Settings: National Intensive Care Unit of Maternity Hospital, Ain Shams University, Cairo, Egypt.

Patients: The study included 30 term asphyxiated neonates (8 with mild, 13 with moderate and 9 with severe HIE) and 20 control neonates.

Intervention: Serum NGAL level was measured within 6 hours after birth using an enzyme linked immunosorbent assay.

Main outcome measures: Patients were subsequently discriminated into AKI (*n*=12) and no-AKI (*n*=18) groups.

Results: The median (Interquartile range) serum NGAL concentration was 95.0 (70.75-180.00) ng/mL in asphyxiated neonates, and 39.75 (6.0-48.0) ng/mL in control neonates; (*P*<0.001). Serum NGAL correlated with HIE severity: mean (SD) was 65.50 (3.77) ng/mL in infants with mild HIE, 115.07 (45.83) ng/mL in infants with moderate HIE and 229.66 (79.50) ng/mL in infants with severe HIE; (*P*<0.01). The median (Interquartiles) serum NGAL level was 182.50 (166.25-301.75) ng/mL in patients with AKI, 74.00 (66.00-78.75) ng/mL in those without AKI; (*P*<0.001). A cutoff value 157 ng/mL for serum NGAL could detect AKI in asphyxiated neonates with a sensitivity of 83.3% and a specificity of 94.4%.

Conclusion: Elevated serum NGAL measured within 6 hours after birth reliably indicates acute kidney injury in asphyxiated neonates.

Key words: Acute kidney injury, Asphyxia, Outcome.

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Acute kidney injury (AKI) is a common consequence of perinatal asphyxia [1] occurring in up to 56% of these infants [2]. The mechanisms of AKI in asphyxiated neonates include diminished renal blood flow because of hypovolemia and hypotension, which can lead to impaired GFR and tubular function [3,4]. In the newborn, the diagnosis of AKI, particularly mild to moderate forms is difficult [5]. Detection of reduced kidney function with a rise in serum creatinine concentration, is an unreliable measure in the acute setting [6]. So the establishment of non-serum creatinine-based AKI diagnostic criteria is crucial for this age group.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25kDa secretory glycoprotein that belongs to the lipocalin family of proteins. Human NGAL was originally isolated from the supernatant of activated neutrophils. Renal expression of NGAL increases dramatically after renal ischemia. This is reflected by the rapid rise in urinary NGAL reported in AKI. NGAL concentration in the serum and urine has been demonstrated to be a sensitive and specific early marker of AKI after cardiac surgery [6,7].

This study was designed to assess serum NGAL level in asphyxiated term neonates within 6 hours of birth, whenever urine sampling is difficult, to evaluate its relation to HIE severity, and its clinical utility for early detection of AKI in these neonates

METHODS

This cohort study was conducted at National Intensive Care Unit of Maternity Hospital of Ain Shams University, Cairo, Egypt, over a period of 10 months from July 2008 till April 2009. The study was approved by the Ethical Committee of the Pediatric Department at Ain Shams University. An informed consent was obtained from one of parents before enrollment of the patients.

Neonates included in the study were ≥37 completed weeks of gestation, appropriate for gestational age. Newborns 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. They were divided into 2 groups:

(*a*) *Patient Group:* It included 30 term neonates with a provisional diagnosis of perinatal asphyxia based on the criteria of American Academy of Pediatrics [8].

(*b*) *Control Group:* It included 20 apparently healthy neonates matched for gestational age, birthweight and postnatal age.

Complete history was elicited from mothers including maternal, obstetric, and perinatal history. Gestational age was calculated based on the date of last menstrual period and confirmed by neonatal examination using the modified Ballard score [9]. Birth weight, sex, and Apgar score at 1, 5 and 10 minutes were recorded. Complete physical examination was done with special emphasis on neurological examination.

Laboratory investigations included complete blood count, C-reactive protein, blood urea nitrogen, serum creatinine (done daily for the first week of life) and serum NGAL (done within the first 6 hours of life), with daily assessment of urine output (24 hours urine output measurement was done by applying plastic collection bag). Oliguria was defined as urine output (<1 mL /kg/ hour). Samples were collected from the control group during sampling for bilirubin measurement within the first 48 hours of life.

Patients were subsequently discriminated, following 48 hours of admission into AKI (*n*=12) and no-AKI (*n*=18) groups. Acute kidney injury was defined as elevation of serum creatinine >1.5 mg/dL for more than 48 hours [10]. Asphyxiated infants were neurologically examined daily over the first two postnatal weeks for the sequential appearance and resolution of various transitory neurological signs and their duration and were subsequently classified according to the Sarnat clinical stages [11] as mild (grade I, *n*=8), moderate (grade II, *n*=13) and severe (grade III, *n*=9) HIE.

Statistical analysis: Statistical analysis was done using SPSS software package, version 15.0, (Ecosoft corporation, USA). Data were expressed descriptively as mean ± standard deviation (SD) for quantitative parametric data and median and interquartile range for quantitative skewed data. Comparison between groups was done using the student *t* test for parametric data and Wilcoxon's rank sum test for skewed data. Correlation study between the different analyzed parameters was done using Spearman's rank correlation coefficient test for skewed data. The diagnostic performance of serum NGAL was evaluated using receiver operating characteristic curve (ROC) analysis.

RESULTS

The demographic and clinical characteristics and laboratory data of studied neonates are listed in ***Table* I**. Neonatal sepsis was excluded in all neonates of the study based on clinical and laboratory criteria (normal blood cell counts, C- reactive protein <6 mg/L and negative blood culture results). We demonstrated a highly significant increase in sNGAL in patient group (median= 95.0 ng/mL, IQ= 70.75-180.00) as compared with control group (median= 39.75 ng/mL, IQ= 6.0-48.0) (*P*<0.001) (***Fig.* 1**).

Serum NGAL correlated with HIE severity. The mean (SD) serum NGAL was 65.5 (3.77), 115.1 (45.83) and 229.7 (79.5) ng/mL in no or mild HIE, moderate HIE and severe HIE groups, respectively. The *P* values were <0.01 for all three comparisons viz. mild *vs.* moderate HIE, moderate *vs*. severe HIE and mild *vs*. severe HIE.

TABLE I CHARACTERISTICS OF THE STUDY CHILDREN

	Patient Group (n=30)	*Control Group (n=20)*	*P value*
Gestational age (weeks)	38.1 (1.29)	38.1 (1.07)	0.67
Birthweight (g)	3250.0 (506.3)	3317.5 (409.5)	0.07
Apgar score 1 min	1.0	8.0	<0.001
5 min	3.0	8.0	<0.001
10 min	6.0	10.0	<0.001
Male	17(55)	8 (40)	0.72
Female	13(45)	12 (60)	
Vaginal delivery*	18(60)	9 (45)	0.72
Cesarean delivery*	12(40)	11 (55)	
Oliguria <1mL/kg/h (*for the first 48 hours*)*	6 (20)	–	
BUN (mg/dL) (*within first 48 hours) Mean*± SD	39.20 (17.67)	16.05 (7.74)	< 0.001
Creatinine (mg/dL) (*within first 48 hours)Mean ± SD*	1.73 (0.42)	0.67 (0.22)	< 0.001

*BUN: blood urea nitrogen; All values in mean (SD); *No. (%).*

A significant positive correlation was found between sNGAL and both serum creatinine and BUN levels determined after 48 hours from birth ($P < 0.05$ and < 0.001, respectively). Patients who were subsequently diagnosed as having AKI, were found to have significantly higher level of sNGAL (median=182.50, IQR=166.25-301.75 ng/mL) compared with those without AKI (median=74.0, IQR=66.00-78.75 ng/mL) ($P < 0.001$) (***Fig.* 2**).

Receiver operating characteristic curve had area under the curve (AUC) of 0.968 with a confidence interval of (1.0-1.0); $P < 0.001$. A serum NGAL cutoff value 157 ng/mL could differentiate asphyxiated neonates with AKI from asphyxiated neonates without AKI**,** with a sensitivity of 83.3%, specificity 94.4%, positive predictive value 85.7% and negative predictive value 92.3% with a diagnostic accuracy 90%.

DISCUSSION

NGAL expression increases greatly in the presence of inflammation and injured epithelia and therefore, NGAL is one of the earliest proteins induced in the kidney after ischemic or nephrotoxic insult. Consequently, NGAL significantly rises in blood and urine soon after AKI [12]**.**

In our study, serum NGAL measured in the first 6 hours of life showed significantly higher values in patient group than control group. Serum levels of NGAL were also 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 serum NGAL and urine NGAL (standardized to urine creatinine and absolute values) than controls at days 1, 3,and 10 [13]. Similarly, the study done by Krawczeski, *et al*. [6] observed that both plasma and urine NGAL concentrations became markedly and significantly higher in both neonatal and non neonatal patients with AKI.

Serum creatinine is not a good marker of renal dysfunction in general and in the neonate there are specific problems associated with it. First, the creatinine concentration reflects the maternal level for up to 72 hours after birth, rendering it unhelpful in the assessment of the neonate in the immediate postnatal period [14]. Second, large changes in the glomerular filtration rate (GFR) occur in the absence of a change in serum creatinine. Moreover, there is significant variability in neonatal GFR/ creatinine values, which change rapidly in the immediate postnatal period as the infant adapts to extrauterine life [15].

Renal failure in the neonate often occurs in the absence of oliguria [3], and a high index of suspicion is required. We depended mainly on serum creatinine levels because only 20% of our studied cases had oliguria in the first day of life while 80% had normal urine output. There was significant positive correlation between NGAL and creatinine and BUN levels in cases group. This comes in agreement with Bachorzewska-Gajewska, *et al.* [16] who found that serum creatinine correlates significantly with both serum and urinary NGAL. Furthermore, serum NGAL steadily increases across groups when stratified according to RIFLE classification. Also in a study done on patients with lupus nephritis, they found that NGAL levels were strongly correlated with renal disease activity but not with extrarenal disease activity score [17].

ROC analysis revealed that sNGAL at a cutoff value of 157 ng/mL, within the first 6 hours of life in asphyxiated neonates, can predict the development of AKI with high

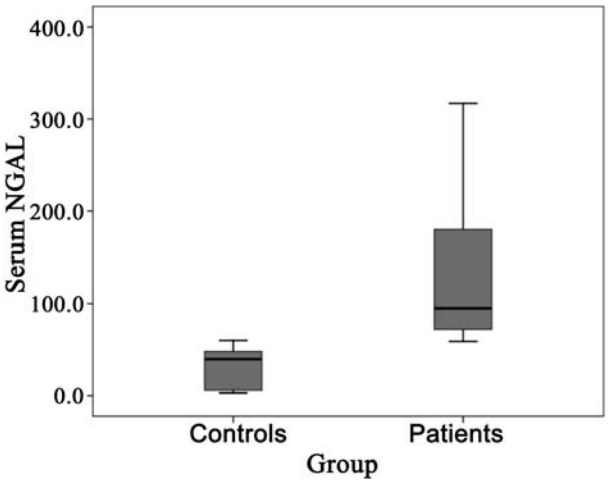

FIG.1 *Serum NGAL levels in patients and control group.*

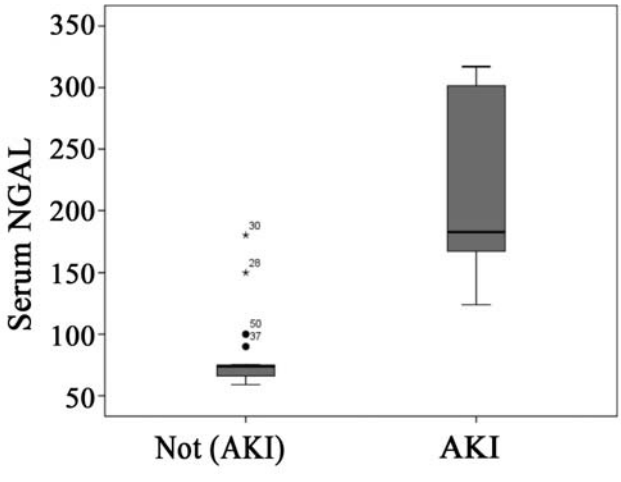

FIG. 2 *Serum NGAL level with and without acute kidney injury.*

WHAT IS ALREADY KNOWN?

- Renal expression of NGAL increases dramatically after renal ischemia.

WHAT THIS STUDY ADDS?

- Serum NGAL could be used within 6 hours of life to predict acute kidney injury in asphyxiated neonates.

sensitivity and specificity. Similarly, another recently published study has demonstrated that serum and urine NGAL could predict AKI in newborns experiencing acute perinatal asphyxia [13]. Another study analyzed ROC curve 2 hours after cardiopulmonary bypass and found that the optimal sensitivity and specificity for plasma NGAL to predict AKI occurred at a value of 95 ng/mL in the neonatal group, and for urine NGAL the value was 185 ng/mL [6]. Mishra, *et al.* [7] that serum NGAL levels at a cutoff value of 139 ng /mL within the first 24 hours of admission to the PICU is highly sensitive for predicting AKI in critically ill children with septic shock with a sensitivity of 86% and a relatively poor specificity of 39%.

We conclude that serum NGAL level is elevated within 6 hrs from birth in term neonates with perinatal asphyxia; in correlation with the evolving HIE severity. High serum NGAL level was significantly associated with the subsequent diagnosis of AKI in these neonates. It could thus be speculated that early measurement of this biomarker in asphyxiated neonates can reliably predict the development of post-asphyxial acute kidney injury.

Contributors: ENM: conceived and designed the study and revised the manuscript for important intellectual content; KSM, ESA: collected the data and drafted the paper; KSM also analyzed the data and wrote the manuscript; MNH: performed the laboratory work. The final manuscript was approved by all authors.

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