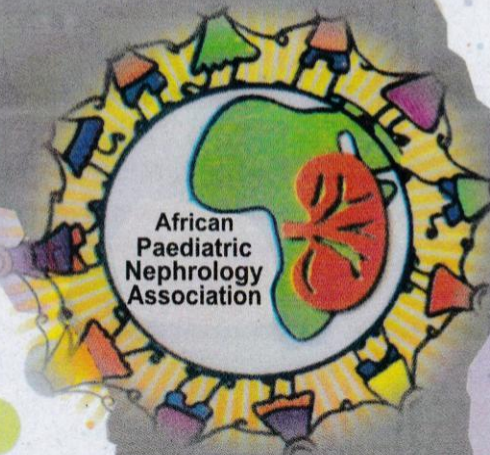


AFRICAN JOURNAL OF PAEDIATRIC NEPHROLOGY



January - June, 2018

Volume 5, Number 1

ISSN: 2354 - 4325

www.afpna.com

Official Journal of
African Paediatric
Nephrology Association
(AFPNA)

Early Detection of Acute Kidney Injury Using Serum Neutrophil Gelatinase-Associated Lipocalin In Asphyxiated Term Neonates in University of Uyo Teaching Hospital, Uyo, Nigeria

Abstract

Background: The increased morbidity and mortality from acute kidney injury (AKI) following birth asphyxia can be reduced by early diagnosis with prompt intervention. Neutrophil Gelatinase-Associated Lipocalin (NGAL) has been demonstrated to be a highly sensitive early biomarker of AKI following birth asphyxia, thus could be utilized for early detection of renal dysfunction for early intervention to prevent long term sequelae. Studies on the clinical utility of serum and urine biomarkers of AKI in neonates are limited in Africa and almost non-existent in Nigeria. This study was therefore carried out to determine the clinical utility of serum NGAL as an early marker of AKI in asphyxiated neonates.

Methods: This was a descriptive cross-sectional study of one hundred and four asphyxiated term neonates and one hundred and four healthy controls matched for gestational age, conducted between July 2015 and February 2016 in the Newborn unit of the University of Uyo Teaching

Hospital, Uyo, Akwa Ibom State of Nigeria. Their serum NGAL levels were measured within 6 hours of life using the highly sensitive Enzyme Linked Immunosorbent Assay (ELISA) technique. Using serum creatinine and urine output criteria, patients were discriminated into AKI and no AKI groups.

Results: The serum NGAL levels in subjects ranged from 30-247ng/ml with mean value of 105.6557.30ng/ml which was remarkably higher than levels in controls that ranged from 16-88ng/ml with mean value being 46.1316.10ng/ml ($p < 0.001$). With serum creatinine criteria, 11.5% of the subjects had AKI while 34.6% had AKI with urine output criteria. Subjects of the AKI group had significantly higher serum NGAL values than those of no AKI group (100ng/ml vs 50ng/ml with $p < 0.001$).

Conclusion: Serum NGAL is a useful biomarker in detecting AKI in asphyxiated neonates within the first 6 hours of life.

Key words: asphyxia, AKI, neonates, NGAL, Nigeria

Corresponding Author (✉)

Prof. Enobong E. Ikpeme
Department of Paediatrics,
University of Uyo Teaching
Hospital, Akwa Ibom State
enobong.ikpeme@gmail.com

Mary P. Udoh FWACP (Paed)
Department of Paediatrics,
University of Uyo Teaching
Hospital, Akwa Ibom State

Introduction

Acute Kidney Injury (AKI) is an abrupt decline in kidney function resulting in inability of the kidneys to regulate fluid and electrolyte homeostasis appropriately.¹ It is associated with primary conditions such as sepsis, perinatal asphyxia, prematurity, metabolic diseases and congenital anomalies of the urinary tract.² It is a common consequence of birth asphyxia.^{3,4} In neonates, the incidence of AKI from birth asphyxia ranges from

30% to 56%³ and its incidence in newborns in Turkey was 40%.² Various incidence rates have been reported in different regions of Nigeria ranging from 3.9/1000⁴ to 34.5/1000 live births⁵ and 35.5%⁶ to 53.4%.⁷

Diagnosis of AKI using serum creatinine (sCr) lacks the capacity, sensitivity and specificity to detect early renal impairment since it varies with age groups, gender, metabolic state, body composition and

excretion by the kidneys⁸ and it reflects maternal levels in the first few days of life.^{9,10} Estimated GFR and oliguria as diagnostic parameters also have limitations as sCr is used in the estimation of the former⁸ and the later is an insensitive predictor as over 50% of AKI in neonates are non-oliguric.^{5,11}

Neutrophil Gelatinase-Associated Lipocalin (NGAL) has been demonstrated to have a high sensitivity in detection of AKI.¹² NGAL is a 25kDa secretory glycoprotein that belongs to the lipocalin family of proteins.¹³ Human NGAL was originally isolated from the supernatant of activated neutrophils. Renal expression of NGAL increases dramatically following renal ischemia and is reflected by a rapid rise in serum and urinary NGAL concentration. This has been demonstrated to be a sensitive and specific early marker of AKI following birth asphyxia.¹³ Schmidt-Ott et al¹⁴ demonstrated that the expression of NGAL rises a thousand-fold in humans and rodents in response to renal tubular injury, and appears so rapidly in urine and blood within few hours thus its usefulness as an early biomarker of kidney injury.¹⁴ Several studies have demonstrated elevated NGAL levels in asphyxiated neonates compared to control groups with markedly increased levels in newborns with AKI following birth asphyxia from the study group in comparison to asphyxiated non-AKI neonates.¹⁵⁻¹⁷ Such studies are limited in Africa and almost non-existent in Nigeria. Early detection of AKI especially in the asymptomatic stage is therefore important in order to institute early measures that may reverse or slow down the progression of kidney injury before significant loss of renal function occurs. This is imperative as the most significant preventive strategy especially as facilities for renal replacement care are limited and expensive in resource-poor countries like Nigeria. This study therefore was designed to evaluate the utility of sNGAL measurement for early detection of AKI in asphyxiated neonates in Nigeria in view of the limitations and drawbacks of the conventional methods that use sCr and UO. Its values in normal newborns will be determined as reference standard for newborns in our locality.

Subjects and Methods

This descriptive cross sectional study was carried out in the Newborn Unit of University of Uyo Teaching Hospital (UUTH), Uyo in Akwa Ibom State of Nigeria over a period of eight months (July 2015 to February 2016). This is a 30-bedded unit where inborn babies requiring intensive care are admitted. Ethical clearance to conduct the study was obtained from the University of Uyo Teaching Hospital Health Research Ethics Committee prior to commencement of the study. Informed consent was obtained from parents/caregivers. The study was conducted on 208 neonates. They were divided into two groups:

Subject group included 104 term neonates with provisional diagnosis of birth asphyxia as defined by Ibe.¹⁸ Control group included 104 apparently healthy term neonates recruited from the labour ward and maternity theatre.

Preterm neonates, neonates with congenital malformations, neonates at risk for sepsis and those whose parents declined consent were excluded from the study.

Gestational age was determined by calculation from date of last menstrual period and/or antenatal ultrasonography and was confirmed by neonatal examination using modified Ballard score.¹⁹ Birth weight, gender, and Apgar score at 1, 5 and 10 minutes were recorded. Asphyxiated neonates were categorized into severe (0-3), moderate (4-5) and mild (6) based on Apgar score at five minutes.

Blood sample (5ml) was collected into plain bottles within six hours of life from each subject, was allowed to clot and centrifuged at 3000 revolutions per minute. The supernatant serum obtained was shared into two, one half was transferred to plain tubes and stored at -80°C until analysis of serum NGAL was done and the second half was used to analyse for serum creatinine. The samples were pooled and analysed in batches of 40 samples every three months. The test for serum NGAL was performed by a qualified chemical pathologist and assisted by the investigator using the Enzyme Linked Immunosorbent Assay (ELISA) Technique with

commercially available reagents provided by Biogene USA KIT 036. In this technique, a monoclonal antibody specific for NGAL had been pre-coated on a micro-plate. Standards and samples were pipetted into wells and any lipocalin-2 present was bound by the immobilized antibody. After washing away the unbound substances, an enzyme linked monoclonal antibody specific for lipocalin-2 was added to the wells. Following a wash step to remove any unbound antibody – enzyme reagent, a substrate solution was added to the wells and colour change developed proportionately to the amount of lipocalin-2 bound in the initial step. The optical density was measured by spectrophotometry method at 450nm wavelength.²⁰ The mean absorbance for each set of duplicate standards, controls and patients' samples was calculated to ensure quality control. A standard curve was created using Sigma Plot 10.0, which generated a good curve fit enabling the NGAL values to be read off. The mean absorbance for each standard concentration was plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis. The optical density value is proportional to the concentration of NGAL.

According to the manufacturer, the ELISANGAL kit is a research-based assay; hence recommended cut-off values were not provided for a positive test. Furthermore, a standard cut-off for NGAL concentration in the detection of AKI has not yet been reported, with all existing studies proposing their own cut-offs.²¹

Serum creatinine estimation was done using modified Jaffe method.²² Urine output was assessed by application of plastic collection bag to the skin by an adhesive patch. The bag was changed six hourly within first 24 hours. AKI was diagnosed when serum creatinine (sCr) > 1.5mg/dl.¹⁷ Oliguria was defined as urinary output < 1.5ml/kg/hour.¹⁷

Statistical analysis: Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20. Data was summarised into tables and graphs as appropriate. Qualitative variables were expressed as numeric values and percentages, while quantitative variables were expressed as mean and standard deviation. Normally distributed continuous variables were compared using student's t-test. The

Wilcoxon rank sum test was used for non-normally distributed continuous variables. Mann Whitney U test was used to test the association between variables. Categorical variables were compared using Chi-square test. P-value < 0.05 was taken to be statistically significant.

Results

Among the asphyxiated babies, 56 (53.8%) were males while 48 (46.2%) were females giving a male: female ratio of 1.2:1. The age of the babies at enrolment ranged from 1 to 5 hours with a mean of 3 hours. A greater percentage of the study population were recruited at 2 hours of life for both the subjects and the controls, 36 (34.6%) each. Details are represented in Table 1.

Twenty eight (26.9%) of the subjects had severe birth asphyxia, 52 (50%) moderate birth asphyxia, while 24 (23.1%) were mildly asphyxiated.

The mean UO (in ml/kg/hr) for the subjects and controls were 1.65 ± 0.68 and 1.6 ± 0.23 respectively and both were comparable ($p=0.67$). A statistically significant difference in the mean sCr of the subjects (0.88 ± 0.46 mg/dl) and controls (0.67 ± 0.34 mg/dl) was demonstrated ($p<0.001$). Similar statistically significant findings were seen with the mean sNGAL (ng/ml) levels between the subjects and controls (105.65 ± 57.30 vs 46.13 ± 16.10 ; $t=10.19$; $p<0.001$). Figure 1 shows the sNGAL levels in the subjects and controls. There was significantly higher values among the subjects (30-247ng/ml) vs the controls (16-88ng/ml). Twelve (11.5%) asphyxiated neonates had AKI based on sCr > 1.5mg/dl while 36 (34.6%) had AKI based on UO criteria < 1.5ml/kg/hr.

Table 2 shows the pattern of serum NGAL in neonates with AKI as determined by sCr and UO criteria. sNGAL level was higher in neonates with AKI using sCr criterion than those with no AKI (143.17 ± 53.42 vs 100.76 ± 56.23). However, the difference in mean was not statistically significant ($p=0.62$). Using the UO criterion, there was a statistically significant ($p<0.001$) higher mean sNGAL value in neonates with AKI (135.57 ± 58.88) compared to neonates with no AKI (86.95 ± 41.94).

The association between sNGAL and AKI using Mann Whitney U test showed median sNGAL value of 100ng/ml in neonates with AKI while neonates without AKI had value of 50ng/ml, showing that most subjects with AKI had sNGAL levels above 100ng/ml.

Figures 2A, B and C showed the actual pattern of NGAL in neonates with AKI as selected by sCr criterion, UO criterion and both criteria respectively. From figure 2A, subjects with AKI had higher median serum NGAL level of 161ng/ml and interquartile range of 106-189ng/ml while subjects without AKI had lower median value of 86.5ng/ml and interquartile range of 55-164ng/ml. In figure 2B, AKI subjects had higher median NGAL level of 168ng/ml and interquartile range of 100-180ng/ml while subjects without AKI had lower median value of 66ng/ml and interquartile range of 46-116ng/ml. Figure 2C, demonstrated median value of 164ng/ml and interquartile range of 100-181ng/ml in AKI subjects and median NGAL level of 64ng/ml and interquartile range of 46-108ng/ml in non-AKI subjects. This demonstrates that patients with AKI using both criteria had serum NGAL levels above 160ng/ml.

Table 3 shows the pattern of sNGAL with age and severity of birth asphyxia in subjects. Most (53) subjects had their sNGAL done at two hours of age. Most (22) subjects (68.8%) with severe birth asphyxia had higher sNGAL levels compared to those with moderate and mild birth asphyxia. The table also shows that 50 (48%) asphyxiated neonates had sNGAL levels above 100ng/ml while 54 (52%) asphyxiated neonates had serum NGAL levels below 100ng/ml.

Table 4 shows the relationship between levels of sNGAL, sCr and UO with the severity of birth asphyxia. Neonates with severe asphyxia had highest mean sNGAL levels (162.96 ± 47.51) compared to those with moderate asphyxia (91.76 ± 48.72) and mild asphyxia (68.87 ± 30.73). The difference in mean was statistically significant (F=33.33; p<0.001). There was also gradual increase in sCr with severity of birth asphyxia with the mean for severe

asphyxia as 1.07 ± 0.48mg/dl for moderate asphyxia 0.83 ± 0.44mg/dl and for mild asphyxia 0.78 ± 0.45mg/dl. This difference in mean was statistically significant (F=3.44; p=0.04). UO was remarkably reduced in subjects with severe asphyxia (1.27 ± 0.64ml/kg/hr) compared to those with moderate asphyxia (1.71 ± 0.68ml/kg/hr) and mild asphyxia (1.96 ± 0.53ml/kg/hr). The difference in mean was statistically significant (F=7.79; p<0.001).

The association between sNGAL and AKI was demonstrated using Mann Whitney U test. The median sNGAL in neonates with AKI was 100ng/ml with interquartile range of 48-176ng/ml. This is significantly higher than median of 50ng/ml and interquartile range of 36-65ng/ml in those without AKI. The p-value of 0.001 was statistically significant.

The mean sNGAL for asphyxiated neonates with AKI using sCr and UO criteria were 143.17 ± 53.42 and 135.57 ± 58.88ng/ml respectively and both were above 100ng/ml

Table 1: Demographic characteristics of study population

	Subjects	Control
Variable	N (%)	N (%)
Gender		
Male	56(53.8)	54(51.9)
Female	48(46.2)	50(48.1)
	104(100)	104(100)
Age (hours)		
1	17(16.3)	25(24)
2	36(34.6)	36(34.6)
3	31(29.8)	32(30.8)
4	13(12.5)	8(7.7)
5	7(6.7)	3(2.9)
	104(100)	104(100)
Gestational Age (weeks)		
18	18(17.3)	17(16.3)
37	34(32.7)	37(35.6)
38	32(30.8)	29(27.9)
39	20(19.2)	21(20.2)
40	104(100)	104(100)

Table 2: Relationship between serum NGAL in neonates with AKI as selected by serum creatinine and urine output criteria

Variables	AKI	Mean?? ± SD	t test	p-value
SCr(mg/dl)	Yes	143.17 ± 53.42	0.50	0.62
	No	100.76 ± 56.23		
UO(ml/kg/hr)	Yes	135.57 ± 58.88	4.60	0.001*
	No	86.95 ± 41.94		

Table 3: Pattern of sNGAL with age and severity of Birth Asphyxia in subjects

Variables	Serum NGAL (ng/ml)					Total n(%)
	1-50 n(%)	51-100 n(%)	101-150 n(%)	151-200 n(%)	201-250 n(%)	
Age (hours)						
1	2(9.1)	4(12.5)	2(11.8)	9(28.1)	0(0)	17(16.3)
2	7(31.8)	10(31.3)	6(35.3)	12(37.5)	1(100)	36(34.6)
3	8(36.4)	12(37.5)	4(23.5)	7(21.9)	0(0)	31(29.8)
4	3(13.6)	4(12.5)	3(17.6)	3(9.4)	0(0)	13(12.5)
5	2(9.1)	2(6.3)	2(11.8)	1(3.1)	0(0)	7(6.7)
	22(100)	32(100)	17(100)	32(100)	1(100)	104(100)
Birth Asphyxia						
Severe	2(9.1)	1(3.1)	2(11.8)	22(68.8)	1(100)	28(26.9)
Moderate	13(59.1)	17(53.1)	13(76.5)	9(28.1)	0(0)	52(50)
Mild	7(31.8)	14(43.8)	2(11.8)	1(3.1)	0(0)	24(23.1)
	22(100)	32(100)	17(100)	32(100)	1(100)	104(100)

Table 4: Comparison of the levels of meanserum NGAL, serum Creatinine, Urinary Output with severity of birth asphyxia

	Mild Mean ± S.D	Moderate Mean ± S.D	Severe Mean ± S.D	F	p value
Serum NGAL	68.87 ± 30.73	91.76 ± 48.72	162.96 ± 47.51	33.33	0.00*
Serum Creatinine	0.78 ± 0.45	0.83 ± 0.44	1.07 ± 0.48	3.44	0.04*
Urinary Output	1.96 ± 0.53	1.71 ± 0.68	1.27 ± 0.64	7.79	0.001*

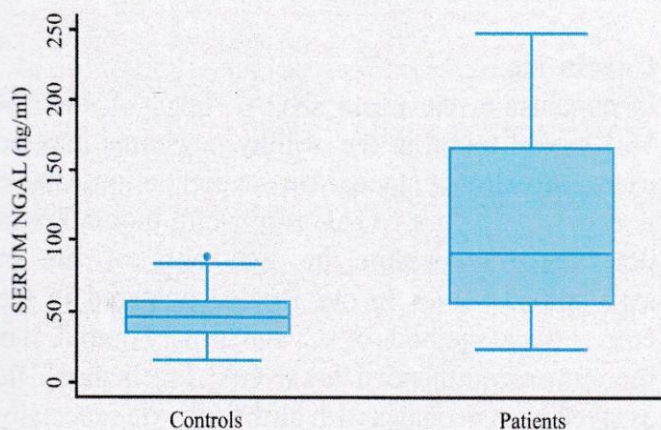


Figure 1: Serum NGAL levels in subjects and control group

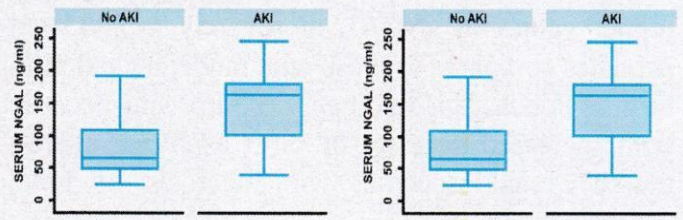


Figure 3A: Graphs by AKI (sCr)

Figure 3B: Graphs by AKI (UO)

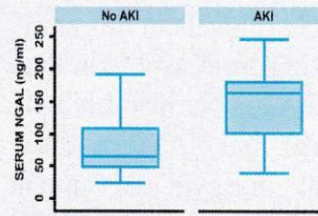


Figure 3C: Graphs by aki

Figure 2: Actual pattern of sNGAL as selected by sCr, UO and both criteria

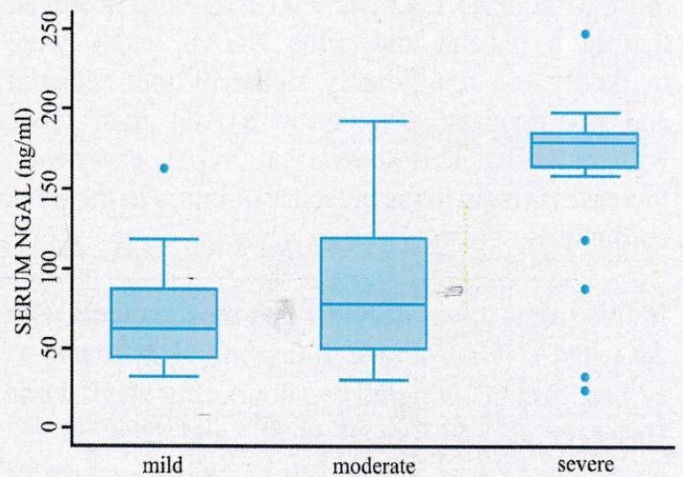


Figure 3: Comparison of serum NGAL levels and severity of birth asphyxia

Discussion

The serum NGAL levels in healthy term newborns in this study of 46.13 ± 16.10ng/ml was comparable to 39.75ng/ml obtained by El-Raggal et al¹⁷ and 55 ± 10.3ng/ml obtained by El-Farghali et al²⁰ both in Egypt in healthy newborn population. However, it was higher than 24.3ng/ml reported by Sumiak et al¹⁵ in Poland. The difference could be explained by the time of sample collection as cord blood sample at birth was used in the Poland study for the NGAL analysis. The value is known to be lower in cord sample as sNGAL levels increase with age.

The increased sNGAL levels with severity of birth asphyxia is similar to the observation in the study done by Krawczeski et al¹³ in children which showed

higher values of sNGAL in severely asphyxiated neonates compared to those with moderate and mild birth asphyxia. This finding is not surprising because with increased severity of birth asphyxia, there's marked renal ischemia with necrosis of renal parenchyma.

The significantly higher sNGAL levels in this study in subjects with AKI following birth asphyxia than asphyxiated babies without AKI is comparable with findings from Krawczeski et al¹³ in USA, Sarafidis et al¹⁶ in Greece, El-Raggal et al¹⁷ in Egypt and Sumiak et al¹⁵ in Poland. Sarafidis et al¹⁶ in their study of AKI biomarkers had significantly higher serum and urine NGAL levels in asphyxiated neonates with AKI than those without AKI. Krawczeski et al¹³ also observed that both plasma and urine NGAL levels were markedly and significantly higher in both neonatal and non-neonatal patients with AKI than those without AKI. This shows that NGAL expression increases greatly in the presence of injury to the renal epithelia.

In this study, it was deduced that most patients with AKI had sNGAL above 100ng/ml. Krawczeski et al¹³ had level of 100ng/ml two hours after insult while Haase et al,¹² El-Raggal et al¹⁷ and Dent et al²³ predicted AKI at higher serum NGAL values of 150ng/ml and above. The higher values obtained in these studies could be due to the number of patients enrolled as well as the methodology used.

AKI demonstrated in 34.6% of the subjects in this study using the urinary output criteria is comparable to about 35% obtained by El-Farghali et al.²⁰ However, Aggarwal et al⁵ reported that oliguria as a bedside indicator of AKI is an insensitive predictor as over 50% of AKI in neonates is non-oliguric. Similarly, Gupta et al¹¹ also showed that most AKI in neonates is non-oliguric. The reliance on oliguria for diagnosis of AKI may cause delay hence the usefulness of NGAL in early diagnosis. In contrast, Devarajan²⁴ reported that following kidney injury, the only sign may be decreased urinary output, but this does not necessarily correlate with the severity of injury. The same criterion of oliguria was applied by Xia-yu et al²⁵ in a retrospective staging of AKI in

asphyxiated neonates. They suggested oliguria as reliable and a sensitive indicator of AKI in neonates. In this present study, there was no difference in urinary output between the subjects and controls. This further shows the unreliability of oliguria as an early predictor of AKI.

Serum creatinine levels obtained in this study within six hours of life were higher in AKI compared to non-AKI group with only 11.5% of the patients having AKI. Previous studies,^{2-3,6,11,26,27} reported prevalence rates of 30-56% using diagnostic cut-off of sCr values of 90-133 μ mol/L at 48 hours of life to define AKI. These higher rates may be explained by the fact that creatinine rises by 24-48 hours after kidney injury whereby 50% of the nephrons may have been lost. This agrees with report by Devarajan²⁴ that sCr within the first day of kidney injury in neonates may remain normal. This is because functional change in sCr is a late consequence of kidney injury and not a marker of injury itself.⁸ Moreover, sCr concentration may not change until 25-50% of kidney function has already been lost, thus it may take days before a significant rise is seen.²⁷

The sNGAL levels increased significantly in neonates with AKI within the first 6 hours of life in this study even when sCr did not rise significantly. Using sCr criterion, AKI was detected in only 11.5% of subjects while sNGAL was able to detect AKI in 50 (48%) of them within 6 hours of life who had sNGAL levels above 100ng/ml. This suggests that sCr is an insensitive marker of AKI in asphyxiated neonates in the first few hours of life.

Conclusion

In conclusion, the mean sNGAL level of 46.13 \pm 16.10ng/ml found in the healthy newborns may be used as a reference standard in our population. Also, it is concluded that sNGAL is a useful biomarker in detecting AKI within the first 6hrs of life in asphyxiated babies in our locale compared to the conventional methods of sCr and urinary output. It is therefore recommended that sNGAL levels should be assayed in all neonates with birth asphyxia especially severe birth asphyxia for early detection of AKI and subsequent appropriate intervention so as to prevent

long term sequelae associated with it.

Acknowledgment

We appreciate all the parents and caregivers who willingly accepted the participation of their babies in this study.

Sources of funding: Self-funded

Conflict of interest: None

Authors' Contributions

Ikpeme EE conceived the idea and supervised the work, read through and corrected the manuscript while Udoh MP carried out the research and wrote the manuscript.

References

1. Noto A, Cibecchini F, Fanos V, Mussap M. Neutrophil Gelatinase Associated Lipocalin and metabolomics: The single biomarker to reveal the metabolome alterations in kidney injury. *Biomed Research Intl* 2013; **10**:1155-1160.
2. Agras PI, Tarcan A, Baskin E. Acute renal failure in neonatal period. *Ren Fail* 2004; **26**:303-309.
3. Durkan AM, Alexander RT. Acute kidney injury post neonatal asphyxia. *J Pediatr* 2011; **158**:29-33.
4. Andreoli SP. Acute renal failure in newborn. *Semin Perinatol* 2004; **28**:112-123.
5. Aggarwal AP, Kumar P, Chowdhary G, Majumdar S, Narang A. Evaluation of renal functions in asphyxiated newborns. *J Trop Pediatr* 2005; **51**:295-299.
6. Anochie IC, Eke FU. Acute renal failure in Nigerian children: Port Harcourt experience. *Pediatr Nephrol* 2005; **20**:1610-1614.
7. Airede AI, Bello M, Weerasinghe HD. Acute Renal Failure in the newborn: Incidence and outcome. *J Pediatr Child Health* 1997; **33**:246-249.
8. Waikar SS, Betensky RA, Bonventre JV. Creatinine as gold standard for kidney injury biomarker studies. *Nephrol Dial Transplant* 2009; **24**:3263-3265.
9. Ashkenazi DJ, Ambalavanan N, Goldstein SN. Acute Kidney Injury in critically ill newborns: what do we know? What do we need to learn? *Pediatr Nephrol* 2009; **24**:265-274.
10. Drukker A, Guiguard JP. Renal aspects of the term and preterm infant: a selective update. *Curr Opin Pediatr* 2002; **14**:175-182.
11. Gupta BD, Sharma P, Bagla JY, Parakh M, Soni J. Renal failure in asphyxiated neonates. *Indian Pediatr* 2005; **42**:928-934.
12. Haase M, Bellomo R, Devarajam P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: A systematic review and meta-analysis. *Am J Kid Dis* 2009; **54**:1012-1024.
13. Krawczeski CD, Woo JG, Wang Y, Michael R, Ma Q, Deverajan P. Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass. *J Pediatr* 2011; **158**: 1009-1015.
14. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DI, Devarajan P et al. Dual action of NGAL. *J Am Soc Nephrol* 2007; **18**:407-413.
15. Surmiak P, Baumert M, Fiala M, Sypniewska K, Walencka Z, Lukomska A et al. Umbilical cord blood Neutrophil Gelatinase-Associated Lipocalin concentration as an early marker of perinatal asphyxia in neonates. *Ginekol Pol* 2014; **85**:424-427.
16. Sarafidis K, Tsepkentzi E, Agakidou E, Diamanti E, Taparkou A, Soubasi V. Serum and urine acute kidney injury biomarkers in asphyxiated neonates. *Pediatr Nephrol* 2012; **27**:1572-1582.
17. El-Raggal NM, Khafagy SM, Mahmoud NH, El-Beltagy SA. Serum neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury in asphyxiated neonates. *Indian Pediatr* 2013; **50**:459-462.
18. Ibe BC. Birth asphyxia and Apgar scoring : A review. *Nig Med Pract* 1990; **20**:111-113.
19. Ballard JL, Khoury JL, Wedig K, Wang L. New Ballard Score: expanded to include extremely premature infants. *J Pediatr* 1999; **119**:417-423.

20. Farghali OG, El-Raggal NM, Mahmoud NH, Zaria GA. Serum Neutrophil Gelatinase-Associated Lipocalin as a predictor of acute kidney injury in critically- ill neonates. *Pak J Biol Sci* 2012; **15**:231-237.
21. Essajee F, Were F, Admani B. Urine NGAL in asphyxiated neonates: A prospective cohort study. *Pediatr Nephrol* 2015; **30**:1189-1196.
22. Lolekha PH, Jaruthunyaluck S, Srisawasdi P. Deproteinization of serum: Another best approach to eliminate all forms of bilirubin interference on serum creatinine by the kinetic Jaffe reaction. *J Clin Lab Anal* 2001; **15**:116-121.
23. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J et al. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: A prospective uncontrolled cohort study. *Crit Care* 2007; **11**:1186-1192.
24. Devarajan P. Neutrophil Gelatinase-Associated Lipocalin: A promising biomarker for acute kidney injury. *Biomark Med* 2010; **4**:265-280.
25. Xiao-yu L, Xin Z, Ying W, Jie D. Retrospective analysis of acute kidney injury in neonates with severe asphyxia. *Chin J Evidence Based Pediatrics* 2011; **6**:275-279.
26. Martin-Ancel A, Garcia-Alix A, Gaya F, Burgueros M, Quero J. Multiple organ involvement in perinatal asphyxia. *J Pediatr* 1995; **127**:786-793.
27. Waikar SS, Bonventre JV. Creatinine kinetics and definition of Acute Kidney Injury. *J Am Soc Nephrol* 2009; **20**:627-679.