K.M Kan
E.E. Ikpeme
O.T. Dixon-Umo

Original Article

Urine Neutrophil Gelatinase Associated-Lipocalin (NGAL) not a useful marker in the Diagnosis of Urinary Tract Infection in Under-five Children in a Tertiary Health Facility in South-South Nigeria.

Corresponding Author (✉)

Enobong E. Ikpeme
Department of Paediatrics, University of Uyo Teaching Hospital, PMB 1136, Uyo, Akwa Ibom State, Nigeria.
enobong.ikpeme@gmail.com
+2348033123336

K.M. Kan
E.E. Ikpeme
O.T. Dixon-Umo
Department of Paediatrics, University of Uyo Teaching Hospital, PMB 1136, Uyo, Akwa Ibom State, Nigeria.

Abstract

Background: The diagnostic gold standard of urine culture in urinary tract infection often requires a long duration of 48-72 hours to obtain results, hence a setback for early intervention. The need for more sensitive rapid diagnostic clues has led to the use of renal biomarkers especially NGAL. NGAL, a novel renal biomarker produced by neutrophils, kidney cells and other body tissues is upregulated by inflammation, ischemia and infection; hence it could be used for early diagnosis as its assay time may be as short as 3.5-4 hours.

Subjects and Methods: This study set out to establish if urine NGAL could be useful in diagnosing UTI among children aged 1-60 months. It was a prospective cross- sectional study of 165 febrile under-five children presenting to the children's outpatient and children's emergency unit of the University of Uyo Teaching Hospital, Uyo, Nigeria. Urine specimen was obtained following strict asepsis by cleaning the genitalia with soap and clean water and genitalia was parted and suprapubic area tapped until child made urine. Urine was collected by clean catch / midstream method into 3 sterile specimen containers for urine culture using cysteine - lactose electrolyte deficient culture media; dip stick urinalysis using combi-11 and urine NGAL was assayed using ELISA method.

Results: Mean urine NGAL values in those with UTI was 26.78 ng/ml versus 47.56 ng/ml in the febrile non-UTI children. The AUC was 0.38 for urine NGAL with a sensitivity and specificity of 41% and 66% respectively at a diagnostic cut-off of 16.11 as obtained from the ROC.

Conclusion: This shows that urine NGAL, as an early renal marker, may be a poor diagnostic tool for early diagnosis of UTI in our locale.

Key words: Neutrophil gelatinase-associated lipocalin (NGAL), not useful, Diagnosis, UTI, Under-fives.

Introduction

Diagnosis of urinary tract infection (UTI), a common childhood bacterial infection is often difficult because of the non-specificity of symptoms.[1–3] Urine culture is the gold standard for diagnosis of UTI.[1–3] It requires 48 to 72 hours to obtain results which may lead to delay in commencement of treatment.[2–4] Rapid tests such as urinalysis for early diagnosis of UTI serve as screening tests but cannot be used to confirm UTI[5] as the nitrite and leucocyte esterase parameters have low sensitivity of 18% though it can be as high as 82%[5-7] coupled with false positive and negative results. This has led to the consideration of renal biomarkers with better sensitivity for possible early diagnosis of UTI and institution of early intervention to prevent complications.[8–14]

Among several renal biomarkers, NGAL has been found to be more useful in UTI diagnosis because of its high sensitivity and specificity ranging from 90-97% and 76-96.6% respectively .[8–11] It is a new generation renal biomarker that has been demonstrated to play a vital role in nephrology especially in acute kidney injury (AKI) and UTI and

it can be assayed within 4 hours.[15–17] NGAL exhibits bacteriostatic function providing the body with innate immunity and is often up-regulated following insults such as ischemia, inflammation and infection.[18-19]Some authors[8–13,] found it useful as its' sensitivity was high whereas others[20–22] did not deem it of any importance because of low sensitivity values.

There is paucity of data in many African countries on the role of renal biomarkers in UTI diagnosis, so in this study we evaluated the utility of NGAL in the early diagnosis of UTI among febrile under-five children in a tertiary hospital in Nigeria.

Subjects and Methods

This prospective cross- sectional study was conducted from June 2017 to September 2017 enrolling 165 febrile under-five children (temperature ⩾ 37.4 °C) presenting at the children's outpatient and children's emergency unit of the University of Uyo Teaching hospital, Uyo.

Inclusion criteria

Children aged 1 month – 60 months with axillary temperature 37.5^{0}C[23] and above were recruited in this study

Exclusion criteria

Any child who had received antibiotics 2 weeks prior to or at onset of fever, was diagnosed with acute renal failure (by sudden deterioration in renal function as evident byhistory of reduced urinary output and sudden swelling of face and legs); with anatomical abnormality in the urinary tract like posterior urethral valves or with chronic diseases like human immunodeficiency virus infection or acquired immunodeficiency syndrome, severe protein energy malnutrition, sickle cell anemia were excluded from the study.

Ethical approval

Approval was obtained from the University of Uyo Teaching Hospital, Uyo Institutional Health Research Ethical Committee (IHREC). Informed consent was obtained from parents/caregivers of all children included in the study.

Urine sample was collected by clean catch / midstream method into 3 sterile specimen containers for urine culture, urinalysis and urine NGAL was assay.

Semi-quantitative estimation of the bacteria colony-forming units (CFU) was by plaiting on Cystein Lactose Electrolyte Deficient (CLED) agar and then incubating at 37^{0}C for 24 hours. It was then inspected macroscopically and also examined under a light microscope. Colony counting was done using a colony counter and diagnosis of UTI was by counting greater than 100,000 CFU/ml. Part of the urine was used for urinalysis to assay for nitrite and leucocyte esterase using Combi-11 strip (TRUE SCREEN COMBI 11A by LIFESANE BIOTECH, U.S.A)
Urine NGAL estimation was by direct enzyme linked immunosorbent assay (ELISA) using Human NGAL Rapid ELISA kit 036RUO (BIOPORTO Diagnostics) according to the manufacturer's instructions. The samples were analyzed in duplicates and the mean of values obtained.

Data Analysis

Data obtained was inputted into Microsoft Excel 2016 (Microsoft Corporation, USA) spreadsheet and analyzed using statistical package for the social sciences (SPSS) version 22. Student t-test was used to compare urine NGAL values in the febrile UTI and febrile non-UTI. Receiver operating curve (ROC) analysis was performed to determine the sensitivity, specificity and diagnostic cut-off values of NGAL. Area under the ROC curve 0.8-1 was regarded to be of good predictive accuracy for NGAL usefulness in UTI diagnosis while AUC 0.5-0.7 was regarded as poor predictors of NGAL usefulness in UTI diagnosis. Statistical significance was established at $p<0.05$.

Results

A total of 165 subjects were recruited for this study of which 88 were males (53.3%) and 77 were females (46.7%) with a male to female ratio of 1.14: 1. Table 1 shows the demographic characteristics of the study

population.

Table 1: Demographic characteristics of the study population

Variable	Frequency	Percentage (%)
Age(months)		
1-12	46	27.9
13-24	36	21.8
25-36	39	23.6
37-48	20	12.1
49-60	24	14.6
Total	**165**	**100.0**
Gender		
Male	88	53.3
Female	77	46.7
Total	**165**	**100.0**

Urinalysis result among individuals with positive culture results

Majority of subjects with UTI did not test positive for nitrite or leucocyte esterase test. Only one individual with *E.coli* had a positive nitrite test, which was not significant and positive leucocyte esterase test present in one child with *Proteus* UTI was also not significant.

Table2: Urinalysis result among individuals with positive culture results

Test	*Staphylococcus aureus*	*Escherichia coli*	*Proteus mirabilis*	*Citrobacter freundii*	Chi square	P value
Nitrite test						
Positive	0	1	0	0	2.3	0.49
Negative	8	6	6	2		
Leucocyte esterase						
Positive	0	0	1	0	2.96	0.39
Negative	8	7	5	2		

Table 3 summarizes the isolated pathogens from the urine of subjects. *Staphylococcus aureus,* a Gram-positive organism, an unusual pathogen was the commonest organism isolated accounting for(34.8%); others were Gram negative organisms: *Escherichia coli* (30.4%), *Proteus mirabilis* (26.1%) and *Citrobacter freundii* (8.7%).

Table 3: Isolated Pathogens from urine of febrile under-fives in UUTH

Isolate	Frequency	Percentage %
Staphylococcus Aureus	8	34.8
Escherichia coli	7	30.4
Proteus Mirabilis	6	26.1
Citrobacter freundii	2	8.7
Total	**23**	**100.0**

Table 4 shows NGAL values in the study population. The mean urine NGAL values in those withUTI was 26.78 ng/ml as against 47.56 ng/ml in the non-UTI febrile children. This difference in NGAL estimation among the study population was not statistically significant at p value of 0.11.

Table 4: NGAL Estimation among the study group

Study group	Mean (ng/ml)	Median	C.I	Range	t (*p* value)
UTI present	26.78	(41.22)	4.91-6.46	6.90-193.00	1.59(0.11)
UTI absent	47.56	(60.06)		2.90-501.30	

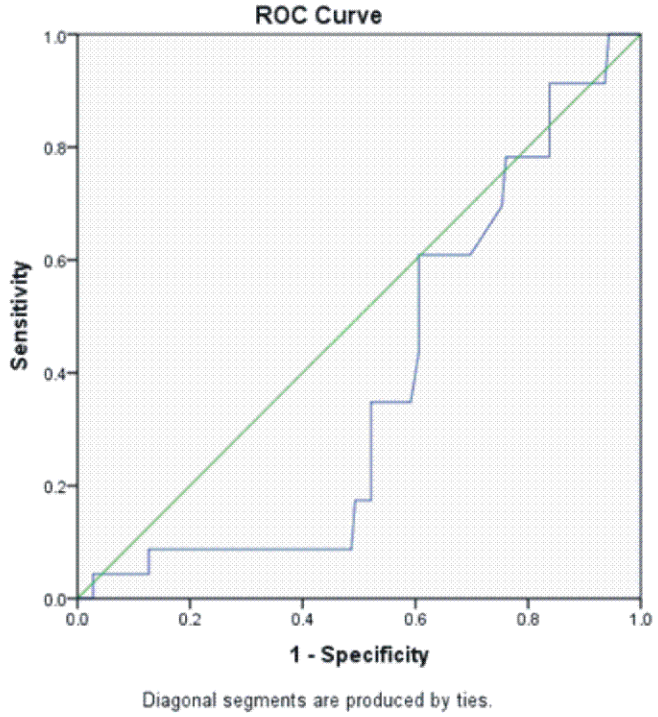
Table 5 evaluates NGAL levels of certain parameters associated with UTI. Leucocyte esterase positive test had higher NGAL levels than nitrite test. When microorganisms were considered, NGAL value was highest in those with UTI due *to E. coli* when compared to other pathogens

Table 5: NGAL values in relation to symptoms, Positive Nitrate, Leucocyte esterase and causative organism

Variable	Mean NGAL Values (ng/ml)	Standard deviation	Range
Nitrite test	16.40	0.91	16.11-17.40
Leucocyte esterase	71.18	40.45	10.80-190.3
S. aureus	15.45	7.03	6.90-21.86
E.coli	58.96	75.28	11.30-193.00
P. mirabilis	12.89	4.10	8.50-16.42
C. freundii	24.20	11.55	12.60-36.54

Figure 1 shows the ROC curve analysis of NGAL The diagnostic cut-off value of NGAL from the area under the curve was maximized at 16.11ng/ml

implying that any individual with an NGAL value of ⩾ 16.11ng/ml should be diagnosed as having UTI. The AUC of 0.38, sensitivity of 44% and specificity of 61% demonstrate NGAL to be a poor diagnostic marker of UTI.



Cut off value= 16.11
Sensitivity= 44%
Specificity= 61%
AUC=0.38 (p =0.07)

Figure 1: Receiver Operating Characteristics (ROC) curve graph of urine NGAL in children with UTI

Discussion

The diagnosis of UTI in children is plagued with non-specific symptoms as well as delay in obtaining culture results hence there is need for an accurate and reliable early diagnostic method with novel renal biomarkers such as NGAL so as to institute early treatment thereby preventing complications. The area under the curve of the receiver operating characteristic (ROC) curve of 0.38 in this study indicates the poor diagnostic accuracy of urine NGAL as a renal biomarker for UTI. This is compared with 0.664 by Safdar [20] who recorded a similar conclusion. Contrary to this, relatively higher AUC of 0.978, 0.992 and 0.942 were obtained by Lubell[9]Yilmaz,[10]and Jung[11] respectively causing them to conclude differently. Authors with high AUC had more than 90% of their isolates being *E. coli*[9–11]suggesting that the type of isolate may influence NGAL levels. The diagnostic cut-off of urine NGAL of 16.11ng/ml in this study would have missed the diagnosis of UTI in more than half of the subjects with the condition as majority of the infections in this study were due to non- *E.coli* organisms.

The low sensitivity and specificity of urine NGAL in this study for diagnosing UTI was comparable with the reports of Safdar[20] and Kim[21] who obtained values of 59.4% and 68.3%,[20] and 70% and 42%[21] respectively. Similarly, in Iran, a low sensitivity of NGAL at 67.4%[22] was of poor diagnostic relevance in UTI pointing to the fact that urine NGAL may not be an efficient biomarker for UTI in these studies. In contrast, other authors reported higher sensitivity and specificity of urine NGAL in febrile UTI subjects giving it diagnostic relevance, as sensitivity and specificity of 96% and 100%,[8] 97% and 76%,[9] 90.2% and 92%[12] respectively. They found *E.coli* as the predominant etiological cause of UTI, which is known to be associated with inflammation leading to pyuria and subsequent NGAL release from the granules of the neutrophils[24–25] compared to non-*E. coli* organisms and Gram positive organisms that do not cause sufficient inflammatory response.[24–26]

Paragas*et al*[27] established that the urinary tract is lined by alpha-intercalated cells which secrete NGAL in the presence of uro-pathogenic organisms causing bacteriostasis.[27] Also the host inflammatory response from UTI causes neutrophil migration and aggregation to the site of infection. These specialized neutrophils also secrete NGAL in response to infection. The presence of bacteria within the urinary tract therefore stimulates the secretion of NGAL, up-regulating its levels and making it a likely marker for early diagnosis of UTI.[28]Some studies in children have shown that Gram positive organisms and

certain non-*E.coli* organisms produce minimal inflammatory response, hence less likely to have pyuria hence minimal NGAL upregulation.[29]

The predominant isolate of *Staphylococcus aureus*, a Gram-positive organism and other non-*E. coli* agents (*Proteus* and *Citrobacter*) may have accounted for the low sensitivity and specificity in the present study, rendering NGAL an unreliable diagnostic marker of UTI.

Mean urine NGAL values in those with UTI was lower than febrile non-UTI children which differs from the report of Safdar[20] who reported mean NGAL of 56 ng/ml in those with UTI as against 24 ng/ml in the non UTI group. Kim[21] also noted urine NGAL values of 19.0 ng/ml in the UTI group to be higher than 14.32 ng/ml in non UTI subjects. The reason for this is not well understood but could be because other subjects with the higher mean NGAL were equally febrile children who may have had other bacterial infections producing an inflammatory response with neutrophil migration as NGAL is known to be upregulated in the presence of infection and inflammation.[26]

Conclusion

This study did not establish the usefulness of urine NGAL in early diagnosis of UTI among febrile under-five children in our locale hence urine NGAL assay may not be an ideal marker for diagnosis of UTI in our environment. We still recommend urine culture as the gold standard for UTI diagnosis. Similar studies should be undertaken in several centers to validate a conclusion on the role of NGAL and other biomarkers in UTI diagnosis in Africa.

Conflict of interest: None declared.

Financial support: No funding.

Acknowledgment

We acknowledge the laboratory scientists of the Chemical Pathology and Medical Microbiology and Parasitology laboratories for analyzing the samples. Our thanks also goes to Mr. Chukwuma Iredu, the statistician who analyzed the data and to all the children for their participation in this study and their parents/guardians who gave consent on their behalf and brought this study to reality..

Authors' contributions

Ikpeme EE conceived the study and supervised the work. Kan KM carried out the work and wrote the manuscript. Dixon-Umo OT also supervised the work and critically read through the manuscript.

References

1. Quigley R. Diagno Lane DR, Takhar SS. Diagnosis and management of urinary tract infection and pyelonephritis. *Emerg Med Clin North Am* 2011; **29**:539-552.
2. sis of urinary tract infections in children, *CurrOpinPediatr* 2009; **21**:194-198.
3. Liaw LC, Nayar DM, Pedler SJ, Coulthard MG. Home collection of urine for culture from infants by three methods: survey of parents' preferences and bacterial contamination rates. *B Med J* 2000; **320**:1312-1313.
4. Owa JA. Urinary tract infections. In: Azubuike JC, Nkanginieme EO (eds). Paediatrics and Child Health in a Tropical Region. 3rd ed. Owerri: African Educational services, 2016; 982-989.
5. Downs SM. Technical report: urinary tract infections in febrile infants and young children. Pediatrics 1999; **103**: e54e.
6. Tsai J-D, Lin CC, Yang SS. Diagnosis of pediatric urinary tract infections. *Urol Sci* 2016; **27**:134-134.
7. Senthilkumar KM, Chandrasekaran C, Anandan H, Balaji N, Sivakumar KV. Screening of Pediatric Urinary Tract Infection using Modified Nitrite Test. *Int J SciStud* 2016; **4**: 42-44.
8. Valdimarsson S, Jodal U, Barregard L, Hansson S. Urine neutrophil gelatinase-associated lipocalin and other biomarkers in infants with urinary tract infection and in febrile controls. *PediatrNephrol* 2017; **32**: 2079- 2087.
9. Lubell TR, Barasch JM, Xu K, Iene M, Cabrera KI, Dayan PS. Urinary Neutrophil Gelatinase-Associated Lipocalin for Diagnosis of Urinary tract infections. *Pediatrics* 2017; e20171090.

10. Yilmaz A, Sevketoglu E, Gedikbasi A, Katyagar S, Kiyak A, Mulazimoglu M, *et al*. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *PediatrNephrol* 2009; **24**: 2387-92.
11. Jung N, Byun HJ, Park JH, Kim JS, Kim HW, Ha JY. Diagnostic accuracy of urinary biomarkers in infants younger than 3 months with urinary tract infections. *Kor J Pediatr* 2018; **61**: 24-29.
12. Lee H-E, Kim DK, Kang HK, Park K. The diagnosis of febrile urinary tract infection in children may be facilitated by urinary biomarkers. *PediatrNephrol* 2015; **30**:123-130.
13. Yim HE, Yim H, Bae ES, Woo SU, Yoo KH. Predictive value of urinary and serum biomarkers in young children with febrile urinary tract infections. *PediatrNephrol* 2014; **29**:2181-2189.
14. Sim JH, Yim HE, Choi BM, Lee JH, Yoo KH. Plasma neutrophil gelatinase associated lipocalin predicts acute pyelonephritis in children with urinary tract infections. *Pediatr Res* 2015; **78**: 48-55.
15. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Barasch J. Dual action of neutrophil gelatinase associated lipocalin. *J Am Soc Nephrol* 2007; **18**: 407-413.
16. Petrovic S, Bogavac-Stanojevic N, Peco-Antic A, Ivanisevic I, Kotur-Stevulijevic J, Paripovic D, *et al*. Clinical application of neutrophil gelatinase associated lipocalin and kidney injury molecule-1 as indicators of inflammation persistence and acute kidney injury in children with urinary tract infections. *Biomed Res Int* 2013; **2013**:1-9.
17. Martensson J, Bellomo R. The rise and fall of NGAL in acute kidney injury. *BloodPurification* 2014; **37**: 304-310.
18. Devarajan P. neutrophil gelatinase-associated lipocalin: a, troponin-like biomarker for human acute kidney injury. *Nephrol* 2010; **15**:419-428.
19. Ichino M, Kuroyanagi Y, Kusaka M, Mori T, Ishikawa K, Shiroki R, *et al*. Increased urinary neutrophil gelatinase associated lipocalin levels in rat model of upper urinary tract infection. *J Urol* 2009; **181**:2326-2331.
20. Safdar OY, Shalaby M, Toffaha WM, Turki AA, Toffaha WM, Ghumaim AH, et al. Neutrophil gelatinase associated lipocalin as an early marker for diagnosis of urinary tract infection Saudi children. *J NephrolTher* 2015; **5**:1-4.
21. Kim BH, Yu N, Kim WK, Yun WK, Lim IS, Kim TH *et al*. Evaluation of Optimal Neutrophil Gelatinase Associated Lipocalin value as screening biomarker for urinary tract infections in children. *Ann Lab Med* 2014; **34**:354-354.
22. Ghesemi K, Esteghamati M, Borzoo S, Parvaneh E. Predictive Accuracy of act infections in children. *Ann Lab Med* 2014; **34**:354-359.
23. Osinusi K, Njinyam MN. Comparison of body temperatures taken at different sites and the reliability of axillary temperature in screening for fever. *Afr J Med and Med Science* 1997; **26**:163-166.
24. Xu SY, Carlson M, Engström A, Garcia R, Peterson CGB, Venge, P. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. *Scand J Clin and Lab Invest* 1994; **54**:365-376.
25. Shaikh N, Shope TR, Hoberman A, Vigliotti A, Kurs-Lasky M, Martin JM. Association between uropathogen and pyuria. *Pediatr* 2016; **138**:e 20160087.
26. Decavele A-SC, Dhondt L, De Buyzere ML, Delanghe JR. Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. *Clin Chem Lab Med* 2011; **49**: 999-1003.
27. Paragas N, Kulkarni R, Werth M, Schmidt-Ott KM, Forster C, Deng R *et al*. α–Intercalated cells defend the urinary system from bacterial infection. *J Clin Invest* 2014; **124**: 2963-2976.
28. Chen L, Zhang W. Kidney alpha-intercalated cells, NGAL and urinary tract infection. *Austin J Hypertens* 2014; **1**:1017-1022.
29. Lubell TR, Schnadower D, Freedman SB, Macias CG, Agrawal D, Kuppermann N *et al*. Comparison of febrile infants with enterococcal and gram-negative urinary tract infections. *Pediatr Infect Dis J* 2016; **35**: 943-948.