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ORIGINAL ARTICLE

Serum and Urine neutrophil gelatinase-associated lipocalin in children with urinary tract infection

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ABSTRACT

Background: Urinary tract infection (UTI) is the commonest bacterial infection in children . Urine culture is the gold standard for diagnosing UTI, but difficulty in urine collection or improperly collected specimen may lead to false positive results of urine culture . the results of urinalysis, such as the presence of leukocyturia, nitrite and leukocyte esterase are used to diagnose UTI in clinical practice, but low sensitivity and specificity of this analysis is an issue . New data show that serum neutrophil gelatinase-associated lipocalin (s NGAL) and urinary NGAL assay can be used for prediction of urinary tract infection (UTI). this study aimed to investigate the relation between urine and serum neutrophil gelatinase-associated lipocalin and UTI in infants in Zagazig University hospitals . .

Methods: A case control study carried out in Pediatric nephrology unit, Faculty of Medicine, Zagazig University Hospitals through a duration of six month starting at august 2018. the study included 48 children between 6 and 12 years divided into three groups arranged as cases with febrile UTI, nonfebrile UTI and healthy control.

all patient were investigated for s NGAL, u NGAL, c-reactive protein, procalcitonin, white blood cell count, urine analysis, urine culture and pelvis-abdominal ultra sound. also, all patient had full detailed history and full examination .

Patient with other infections, manifestations of chronic renal failure, manifestations of liver cell failure, evidence of obstructive nephropathy and with vesicoureteral reflux were excluded.

Results: the study showed that, there was significant increase in level of serum NGAL in patients with febrile and non-febrile UTI in comparison to healthy control group with mean sensitivity 100%, specificity 75%, positive predictive value 66.7%, negative predictive value 100% and cutoff of serum NGAL in prediction of presence of febrile UTI in the studied patients is ≥ 140(pg/ml) with area under curve 0.906 .There was significant increase in level of urine NGAL in patients with febrile UTI in comparison to patients with nonfebrile UTI and healthy control group with sensitivity 81.2%, specificity 50%, positive predictive value 76.5%, negative predictive value 57.1% and cutoff of urine NGAL in prediction of presence of febrile UTI in the studied patients is ≥ 129(pg/ml) with area under curve 0.774 .

Conclusions: Serum NGAL had sensitivity and specificity in prediction of UTI in studied patients more than urine NGAL..

Keywords: UTI, serum NGAL, urine NGAL.

INTRODUCTION

Urinary tract infection (UTI) is the commonest bacterial infection in children [1]. The results of urine analysis, such as leukocyturia, increase the level of nitrite and leukocyte esterase are used to diagnose UTI in clinical practice, but they have low sensitivity and specificity [2]. collection of urine sample is difficult and also, urine collection under improper aseptic conditions may lead to false results of urine culture and urinalysis in infant [3]. For diagnosis of UTI, urine culture was done , but results need at least 2 days for a complete detection

of bacteria [4]. also, pus cells in urine in infants may be a sign of other inflammatory changes, including perineum, vaginitis, diarrhea, synechia, or phimosis. Sterile pyuria may occur in noninfectious conditions such as renal stones, congenital abnormalities of kidney and urinary tract (CAKUT), recent history of urological intervention, dehydration, and high fever of external causes [5].

Early diagnosis of UTI is needed , because any delay in diagnosis and treatment lead to severe renal parenchymal affection and increase the risk of chronic renal failure [6]. so, new blood and urine biomarkers are needed for an early detection of UTI in children [7]. New data show that serum neutrophil gelatinase-associated lipocalin (s NGAL) and urinary NGAL assay can be used for prediction of UTI [7].

Neutrophil gelatinase-associated lipocalin (NGAL) is specialized in binding and transporting small hydrophobic molecules including iron [8]. NGAL has been demonstrated to be an early biomarker in acute renal injury after cardiopulmonary bypass, major cardiac surgery, elective cardiac catheterization and angiography, hemolytic uremic syndrome, and kidney transplantation. NGAL is also a biomarker for chronic kidney diseases, such as immunoglobulin A (IgA) nephropathy, membranous and membranoproliferative glomerulonephritis, autosomal dominant polycystic kidney disease, and pediatric LN [9]

Neutrophil gelatinase-associated lipocalin (NGAL) plays an important role in innate antimicrobial immune system [2].

The main effect of NGAL on gram-negative bacteria is binding and sequestration of iron-loaded bacterial siderophores, which prevents bacterial iron uptake and growth. NGAL also modulates neutrophils functions like maturation, adhesion, phagocytosis and bacterial killing. It also acts as chemoattractant for neutrophils. Neutrophils and epithelial cells of several tissues including kidney, liver, lungs, and colon have a very low concentration of NGAL in healthy persons [10].

METHODS

This case control study was conducted at Pediatrics Nephrology Unit, Faculty of Medicine, Zagazig University Hospitals through a duration of six month starting at august 2018. A total sample of 48 children, divided into three groups 16 in each group.

Group 1: children has febrile UTI between the age of 6 years and 12 years.

Group 2: age and sex matched children who has non febrile UTI

Group 3: age and sex matched healthy children who attend outpatient clinics for routine care.

Inclusion criteria including that patient had UTI and the age of each group ranged between 6 and 12 years old. The diagnosis of UTI was done by urine culture and urine analysis.

Patient with other infections, manifestations of chronic renal failure , manifestations of liver cell failure, evidence of obstructive nephropathy and with vesicoureteral reflux were excluded.

All patients was subjected to full history and stress on history of recurrent UTI, family history of renal stones , history of UTI symptoms like dysuria, frequency, urgency, nocturnal enuresis and fever without obvious sources. also, full general examination was done and stress on body temperature, throat examination, chest auscultation to detect any focus of fever and blood pressure. abdominal examination was done and stress on palpation on renal angle and on suprapubic area to detect any tenderness and palpation of abdomen to detect any organomegaly . patient had investigations like : urine analysis, urine culture , serum C-reactive protein (CRP) level, leucocyte count in blood , procalcitonin (PCT)and urine and serum neutrophil gelatinase –associated lipocalin(NGAL).

Procedures of urine and serum neutrophil gelatinase associated lipocalin(NGAL) : The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human neutrophil gelatinase -associated lipocalin (NGAL) in samples. NGAL was added to monoclonal antibody enzyme which was pre-coated with human NGAL monoclonal antibody then incubated . NGAL antibodies labeled with biotin was added and combined with streptavidin -HRP to form immune complex . then the incubation was carried out and the sample was washed again to remove the uncombined enzyme . Then chromogen solution A,B was added. the color of the liquid changed into blue. By the effect of acid , the color finally becomes yellow. The chroma of color and the concentration of human NGAL of sample were positively correlated.

Ethical Declaration:

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans

STATISTICAL ANALYSIS:

Data were coded, entered, checked and analyzed using SPSS version 20.0 (Statistical Package for the Social Sciences). Data were presented as Mean

± Standard deviation (SD) for quantitative variables, numbers and percentage for categorical variables. T test was used for independent samples. Chi square test (χ^2) when appropriate. the level statistical significance was set at P<0.05. highly significant difference was present if P value≤0.001

RESULTS

Demographic data of both case and control groups were presented in table (1). Mean age of febrile patient with UTI was 9.5±1.5 while mean age of a febrile group was 9.2±2 while mean age of control group was8.7±1.2 . females represent 62.5% of febrile patient , 56.3% of afebrile group and 50% in control group.

Mean serum NGAL was 1194.89±1086.419 in febrile patient with UTI , 551.69±871.74 in afebrile patient with UTI and 96.02 ± 16.57 in control group with statistically significant p<0.05. while Mean urine NGAL was 159.89±35.34 in febrile patient with UTI and , 132.31 ± 17.34 in afebrile patient with UTI and 116.75±33.67 in control group with statistically significant (p<0.05).(table 2)

The correlation coefficient between pus in urine and urine NGAL was 0.462 while correlation coefficient between pus in urine and serum NGAL was 0.592. the correlation coefficient between urine NGAL and total leukocytic count was 0.623 while correlation coefficient between serum NGAL and total leukocytic count was 0.628. the correlation coefficient between C-reative protein and urine NGAL was 0.611 while correlation coefficient between C- reative protein and serum NGAL was 0.717.The correlation coefficient between procalcitonin and urine NGAL was 0.777 . The correlation coefficient between procalcitonin and serum NGAL was 0.811 Table (3).

Mean serum NGAL was 161.5±45.47 in Acinetobacter urine culture while mean urine NGAL was 25.5±16.26 in same culture .mean serum NGAL was 1222.9 ±1264.58 in E.coli urine culture while mean urine NGAL was 149.67±37.96 in same culture. In Gram negative bacilli culture, mean serum NGAL was 572.67±539.68 and mean urine NGAL was 134±16.37. in klebsiella urine culture, mean serum NGAL was 895.15±840.48 while mean urine NGAL was 154.48±22.72. mean serum NGAL was 169.55±37.55 in proteus urine culture while mean urine NGAL was 125.6±14.99. mean serum NGAL was 199.2 in pseudomonas urine culture and mean urine NGAL was 128 in same culture. in Staph urine culture , mean serum NGAL was 833.75±847.51and mean urine NGAL was 59±31.54. in Streptococci urine culture, mean serum NGAL was 107 ,but mean urine NGAL was 144 in same culture. Table (4)

Figure (1): Scatterdot graph showing significant positive correlation between serum NGAL(pg/ml) and urine NGAL(pg/ml) among the studied participants.

Figure (2): ROC curve showing performance of serum NGAL in prediction of febrile UTI in the studied patients.

Figure (3): Scatterdot graph showing significant positive correlation between serum gelatinase-associated lipocalin(pg/ml) and procalcitonin(ng/ml) among the studied participants)

Table (1): Comparison between the studied groups regarding demographic characteristics:

	Febrile UTI group (16)	Non-febrile UTI group (16)	Control group (16)	X2	P
	N (%)	N (%)	N (%)		
Gender: **Male** **Female**	 **6 (37.5)** **10 (62.5)**	 **7(43.7)** **9(56.3)**	 **8(50)** **8 (50)**	 **0**	 **1**
Age(years) **Mean SD** **Range**	 **9.5±1.5** **3 – 11**	 **9.2 ± 2** **6 – 11**	 **8.7±1.2** **6 – 12**	 **F** **1.7**	 **0.194**

F one way ANOVA

Table (2) Comparison between the studied groups regarding serum and urine NGAL:

	Febrile UTI group	Non-febrile UTI group	Control group	KW	P
SerumNGAL(pg/ml) Mean ± SD Range	 1194.89±1086.41 193.3 – 3332	 551.69±871.74 103 – 2944	 96.02 ± 16.57 73 - 138	32.24	<0.001**
Pairwise Comparison	P1 0.075	P2 0.002*	P3 <0.001**		
Urine NGAL(pg/ml) Mean ± SD Range	 159.89±35.34 114 – 255	 132.31 ± 17.34 108 – 165	 116.75±33.67 61 - 164	F 8.536	0.001**

*p<0.05 is statistically significant
P1 the difference between febrile UTI and non-febrile UTI groups
P2 the difference between non-febrile UTI and control groups
P3 difference between febrile UTI and control groups

Table (3) Correlation between serum, urine neutrophil gelatinase-associated lipocalin and laboratory data of the studied participants:

	Serum NGAL(pg/ml)				Urine NGAL(pg/ml)		
	Mean ± SD	Median	KW	p	Mean ± SD	F	p
Acinetobacter	161.5±45.47	161.15	4.619	0.706	25.5±16.26		
E.coli	1222.9±1264.58	496			149.67±37.96		
Gram (-ve) bacilli	572.67±539.68	441			134 ± 16.37		
Klebsiella	895.15±840.48	863.3			154.48±22.72		
Proteus	169.55±37.55	169.55			125.6±14.99		
Pseudomonas	199.2	199.2			128		
Staph	833.75±847.51	588			59±31.54	0.479	0.84
Streptococci	107	107			144		

*r correlation coefficient
*p<0.05 is statistically significant
CRP: c reactive protein
TLC: total leucocytic count

Table (4): Relation between serum, urine neutrophil gelatinase-associated lipocalin and bacteria incriminating in UTI of the studied participants:

	Serum neutrophil gelatinase-associated lipocalin		Urine neutrophil gelatinase-associated lipocalin	
	r	**P**	**r**	**P**
Serum neutrophil gelatinase-associated lipocalin			0.598	<0.001**
urine neutrophil gelatinase-associated lipocalin	0.598	<0.001**		
Pus	0.592	<0.001**	0.462	0.008*
TLC	0.628	<0.001**	0.623	<0.001**
CRP	0.717	<0.001**	0.611	<0.001**
Procalcitonin	0.811	<0.001**	0.777	<0.001**

NGAL: neutrophil gelatinase-associated lipocalin

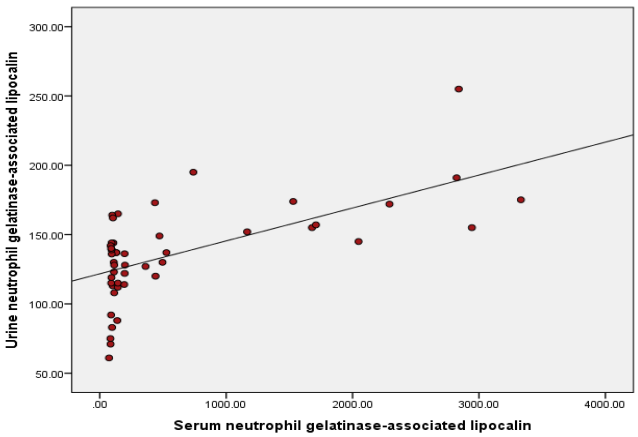

Figure (1) Scatterdot graph showing significant positive correlation between serum NGAL(pg/ml) and urine NGAL(pg/ml) among the studied participants

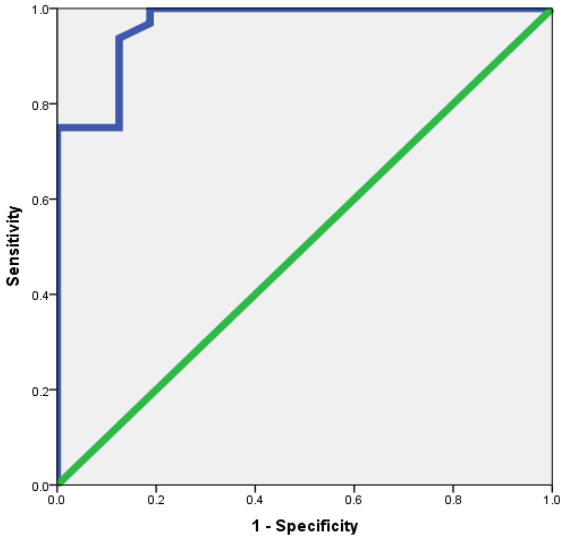

Figure (2): ROC curve showing performance of serum NGAL in prediction of febrile UTI in the studied patients.

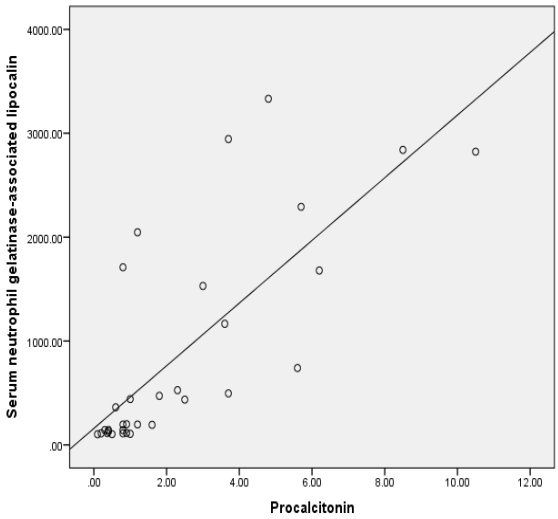

Figure (3): Scatterdot graph showing significant positive correlation between serum gelatinase-associated lipocalin(pg/ml) and procalcitonin(ng/ml) among the studied participants

DISCUSSION

Urinary tract infection (UTI) is the commonest bacterial infection in children[1] . New data show that serum neutrophil gelatinase-associated lipocalin (s NGAL) and urinary NGAL assay can be used for prediction of urinary tract infection (UTI)[7].

In this study, there was significant increasing in level of serum NGAL in febrile and non-febrile UTI in comparison with the level of serum NGAL in healthy control group .that was in agreement with .**Krzemień et al**[11]who showed that serum NGAL was an excellent marker for the diagnosis of febrile UTI. it also had high sensitivity and specificity. It also was in a line with **Munilakshmi et al [12]** who showed that investigations such as NGAL and CRP played an important role in early diagnosis of UTI in parallel to microbiological investigations.

In this study there was significant increasing in level of urine NGAL in febrile UTI in comparison with level of urine NGAL in both non febrile UTI and healthy groups. It was in agreement with **Yilmaz et al** [2] who showed that the level of urinary NGAL increased in children with UTI and that it was an early predictive biomarker of infection. It also had high sensitivity and specificity in the absence of acute renal failure and chronic kidney disease. In contrast to **Osama Safdar et al[13]** who reported that Urinary NGAL was a poor biomarker for the diagnosis of febrile UTI. other investigations were needed with larger groups of patient to confirm the results of this study.

This study showed that there was positive correlation between serum and urine NGAL and pus cells in urine, total leucocytic count in blood, C reactive protein and serum procalcitonin. That was in agreement with **Hyung Eun Yim et al[14]** study showed that NGAL levels were significantly correlated with serum levels of WBCs, CRP, and Cr, as well as the duration of fever.it also was in a line with **Smertka et al** [15] study showed that serum and urinary NGAL concentrations were significantly correlated with inflammatory markers like CRP and PCT. In contrast to **Yilmaz et al [2]** who showed that Urine NGAL was not correlated to serum CRP, urine nitrite test, pyuria, urine pH and specific gravity. Also , leukocytosis in serum and leukocyte esterase reaction in urine were not correlated to the level of urinary NGAL.

In this study, serum NGAL had sensitivity and specificity in prediction of UTI in studied patients more than urine NGAL. It was in agreement with **Krzemień et al [11]** who showed that serum NGAL was an excellent marker for the early diagnosis of febrile UTI . serum NGAL had sensitivity and specificity higher than those of urine NGAL. it was against **Nishida et al [16]** who showed that urinary NGAL level was a better biomarker for chronic renal diseases in children than serum NGAL. Also, it was in contrast to **Smertka et al [15]** that showed that the level of serum and urinary NGAL did not reflect the severity of inflammatory reactions. Their values were not solely a marker of kidney injury in septic newborns.

LIMITATIONS OF THE STUDY

The limitation of study were small sample size and difficulty to obtain clean urine sample for accurate analysis

CONCLUSION

there was statistically non-significant difference between the studied groups regarding age or gender. Also, there was non-significant negative correlation between age and serum or urine neutrophil gelatinase-associated lipocalin The study results showed that there was significant positive correlation between serum and urine neutrophil gelatinase-associated lipocalin and all of Pus in urine, total leucocytic count, C reactive protein and serum procalcitonin. there was statistically non-significant difference between type of bacteria and either serum or urine NGAL in the studied patients .

there was significant increasing in level of serum NGAL in febrile and non febrile UTI in comparison with the level of serum NGAL in healthy control group. There was significant increasing in level of urine NGAL in febrile UTI in comparison with level of urine NGAL in both non febrile UTI and healthy groups. Serum NGAL has high sensitivity and specificity in UTI prediction more than urinary NGAL.

RECOMMENDATIONS

The study recommended that early screening of UTI must be done for all children to decrease morbidity and mortality. Also, serum and urine NGAL should be used as screening tools for UTI in children and serum NGAL is more specific and sensitive than urine NGAL in diagnosis of UTI.

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