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Urinary Neutrophil Gelatinase Associated Lipocalin and Interleukin-18 as Early Predictors of Kidney Injury in Neonates

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Abstract

Background: Early detection of renal affection in neonates is vital for early treatment and prevention of dangerous complications, so the using of some markers like urinary neutrophil gelatinase associated lipocalin (uNGAL), interleukin-18 (uIL-18) is very important as the commonly used detectors as creatinine are very late.

Patient and methods: This study included 80 neonates and was carried out at neonatal intensive care unit (NICU), pediatrics department, Tanta university hospital, Egypt from 6/2013 to 6/2015. Group 1 (kidney injury-patient group): This group included forty neonates Group 2 (Control Group): This group included forty healthy neonates from outpatient clinic. uNGAL, uIL-18 and serum creatinine were assessed in 80 neonates at day 1 and day 3 of neonatal intensive care unit admission who had renal affection later proved by BUN serum creatinine.

Results: Means of uNGAL, uIL-18 and serum creatinine in the day 1 of admission in cases and control groups were (30.94 ± 10.05 *vs* 6.7 ± 4.49), (11.61 ± 9.00 *vs* 1.1.98 ± 1.18) and (0.64 ± 0.17 *vs* 0.59 ± 0.19) respectively with p-value <0.05 which was significant in uNGAL and uIL-18 only.

Conclusions: uNGAL and uIL-18 are considered simple and accurate markers in early detection of renal impairment in neonates which are better than serum creatinine

Keywords: Neutrophil gelatinase associated lipocalin; Interleukin-18; Neonates; Kidney

Introduction

Kidney injury in neonates is a serious neonatal problem, particularly in neonatal sepsis and hypoxia that leads to failure of the kidneys to excrete wastes and maintain fluid balance [1]. Early detection of renal affection in neonates is essential for early management and prevention of serious complications. The old biomarkers for the detection of kidney impairment are serum creatinine, and blood urea nitrogen (BUN) which are late markers causing delaying in management of kidney impairment with bad prognosis [2,3].

Serum creatinine usually rises only 24-36 hours after renal impairment and it may also reflect the maternal renal state in this very early stage of life, therefore [4].

Some of the earliest non-invasive biomarkers of kidney impairment which could be used in neonates are uNGAL and uIL-18 [5].

NGAL is a 25 kDa protein of 178 amino acids, covalently bound to gelatinase from human neutrophils [6]. NGAL-2 was recently identified as one of the earliest proteins produced by the kidney after ischemic injury and this is reflected by the rapid rise in urinary NGAL-2 reported in AKI. NGAL-2 concentration in the serum and urine has been demonstrated to be a sensitive and specific early marker of AKI after cardiac surgery [7].

Urinary interleukin-18 is also being promoted as an early non-invasive biomarker of kidney impairment in neonates. Interleukin-18 is an18 kDa pro-inflammatory cytokine initially synthesized in its inactive form (24 kDa) and subsequently cleaved by caspase-1 in the epithelial cells of the proximal tubules into its active form [8]. Urinary IL-18 performed best as a predictor of AKI at 12 h compared to other time points. Both uNGAL and uIL-18 were independently associated with duration of AKI [9].

The present study aimed to detect the role of uNGAL and uIL-18 in detecting early renal impairment in neonates to help in early and accurate diagnosis with early management to prevent serious complications.

Patients and methods

This study was approved by the ethical committees of faculty of medicine, Tanta University, Egypt. All chemicals unless otherwise described were purchased from sigma (Sigma, St Louis, USA). All chemicals and solvents were of high analytical grade.

Study design

We prospectively enrolled in this study 80 neonates who were admitted to neonatal intensive care unit at Tanta University Hospital from 6/2013 to 6/2015. Informed consents of the parents after a complete description of the study were obtained. Participants are divided in to 2 groups. Group 1 (kidney injury-patient group): This group included 40 neonates: who developed renal impairment later proven by BUN and serum creatinine. Group 2 (Control Group): This group included 40 healthy neonates without renal impairment.

All participants subjected to; detailed medical history:

Clinical examination including: Vital signs (pulse-temperature-

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		Cases(n=40)		Controls(n=40)		Total		Chi-square	
		N	%	N	%	N	%	X^2	P-value
Gender	Female	18	45.00	12	30.00	30	37.50	1.923	0.166
	Male	22	55.00	28	70.00	50	62.50		
Mode of delivery	NVD	14	35.00	16	40.00	30	37.50	0.209	0.644
	CS	26	65.00	24	60.00	50	37.50		
NVD=Normal Vaginal Delivery; CS=Caesarian section; n=number									

Table 1: Shows the gender and mode of delivery of all newborns.

	Cases (n=40)		Control (n=40)			
	Mean	SD	Mean	SD	t. test	P-value
Gestational age (week)	38.4	1.2	38.5	1.2	0.297	0.767
Birth weight(gm)	3115.7	46.7	3129.9	57.5	1.043	0.299
Apgar at 1 min	6.2	0.8	8.4	0.8	7.100	<0.001*
Apgar at 5min	7.3	0.7	9.5	0.7	8.115	<0.001*
Hematocrit (%)	43.4	10.6	53.8	7.1	5.335	<0.001*
* p value <0.05 significant						

Table 2: Demographic characteristics of cases and control.

		The first day (Day 1)			
		Cases	Control	t test	p value
uIL-18 (ng/ml)	Range	2.50-21.7	0.75-3.25	6.705	0.001*
	Mean ± SD	11.61 ± 9.00	1.98 ± 1.18		
uNGAL (ng/ml)	Range	20.4-41.7	2.20-10.20	12.387	0.001*
	Mean ± SD	30.94 ±_10.05	6.7 ± 4.49		
Creatinine (mg/dl)	Range	0.55-0.75	0.5-0.70	1.237	0.219
	Mean ± SD	0.64 ± 0.17	0.59 ± 0.19		
* p value <0.05 significant					

Table 3: Shows mean of uNGAL, uIL-18 and serum creatinine in the day 1 of admission.

blood pressure-respiratory rate), clinical picture of primary illness, and clinical picture of renal impairment.

Exclusion criteria: Abnormal maternal renal function, history of maternal renal disease, neonates with congenital anomalies, multiple malformations, and chromosomal abnormalities, failure of urine sampling to be obtained before 48 hours of birth, babies who did not develop renal impairment and babies who died during the study.

Blood sampling: Blood samples were aseptically collected sterile tubes. The collected blood was used for serum separation (centrifuged at 3000 rpm for 15 minutes at 4°C) and stored at -80°C for further analysis.

Urine sampling: Spontaneous voided urine samples were obtained using urine collection bags or squeezing urine from cotton balls placed in diapers. Urine samples immediately centrifuged at 3000 × g for 10 min at 4°C to obtain the supernatant which stored at -80°C until testing. All pathological samples showing proteinuria, hematuria, bacteriuria or abnormal sediments were excluded.

Methods

Each group will be subjected to the following:

1. Serum creatinine was estimated using commercial kits (Diamond Diagnostic, Egypt)

2. Urinary NGAL and IL-18 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Sunred Biological Technology Co. Ltd, Shanghai, China and R and D Systems Inc., Minneapolis, MN, USA respectively) [10,11]. According to manufacturers' instructions and read on microplate reader (Stat Fax® 2100, Fisher Bioblock Scientific, France), at 450 nm with correction wavelength set at 570 nm.

Results

Eighty newborns were included in the study (50 males and 30 females), subdivided into cases and controls. There is no significance between the two groups (P>0.05) as regard the gender and mode of delivery as shown in Table 1.

The mean and standard deviation of the gestational age (weeks), birth weight (gm), Apgar score at 1 min, Apgar score at 5 min and hematocrit value (%) of cases and control are presented in Table 2. There was no significant difference between the demographic characteristics except Apgar score and hematocrit value (%) that showed statistically significant difference (P-value <0.05).

Means of uNGAL, uIL-18 and serum creatinine in the day 1 of admission are presented in Tables 3 and 4 which show significant difference between cases and controls as regard to urinary NGAL-2 and IL-18 and non-significant difference between cases and controls as regard creatinine while Table 4 shows mean of uNGAL, uIL-18 and creatinine in the day 3 of admission among the study groups in which significant difference between cases and controls as regard to uNGAL and uIL-18 and significant difference between cases and controls as regard creatinine were observed.

Significant positive correlation (+ ve) between uNGAL and uIL-18 was detected as shown in Figure 1. Sensitivity, specificity, predictive values and cutoff values of uNGAL-2 and uIL-18 and serum creatinine are presented in Table 5 which detected that uIL-18 is more sensitive than uNGAL-2 and creatinine and that uNGAL are more sensitive than

serum creatinine and finally Figure 2 shows receiver-operating curve (ROC) for uNGAL-2 and uIL-18 and serum creatinine.

Discussion

Renal impairment in neonates is characterized by a rise in the serum concentration of urea and creatinine and by failure of the kidney to maintain fluid and electrolyte homeostasis properly [12,13].

BUN and creatinine are late, nonspecific and non-sensitive markers in early detection of kidney impairment in neonates. A delay in diagnosis prevents early management decisions, including administration of therapeutic agents. Early biomarkers of AKI will facilitate earlier diagnosis and specific preventative and therapeutic strategies, ultimately resulting in fewer complications and improved outcomes [14,15].

In the present study, cases had significantly elevated uNGAL, as well as uIL18. Serum creatinine (Cr) is the most commonly used clinical measure of renal function, not injury, so it is a poor marker for diagnosis of AKI. Serum Cr may not increase until about 25% to 50% of renal function is lost [15]. uNGAL as well as IL18 are of significant clinical importance as early predictors of renal impairment, increased levels of these markers are supposed to indicate early tubular damage in cases of kidney injury [16]. In agreement with our study there were many studies which were done on children and revealed that NGAL-2 and IL-18 rises significantly in patients with AKI if compared to controls, furthermore, the rise in NGAL-2 and IL-18 in these studies occurs at 24 to 48 h before the rise in serum creatinine and so these markers could be used as early predictors of AKI in neonates [17-24]. According to our results, neonates had significantly increased levels of uNGAL and uIL-18 which are of significant clinical importance as early predictors of AKI and can predict the development of AKI with high sensitivity and specificity.

		The third day (Days 3)			
		Cases	Control	t-test	p value
uIL-18 (ng/ml)	Range	3.99-26.7	0.79-3.22	6.613	0.001*
	Mean ± SD	14.61 ± 12.03	2.00 ± 1.19		
uNGAL (ng/ml)	Range	40.4-70.97	2.20-10.20	11.879	0.001*
	Mean ± SD	55.94 ± 15.75	6.7 ± 4.49		
Creatinine mg/dl	Range	1.4-1.6	0.4-0.7	9.258	0.001*
	Mean ± SD	1.48 ± 0.61	0.54 ± 0.20		
* p value <0.05 significant					

Table 4: Shows mean of uNGAL, uIL-18 and serum creatinine in the day 3 of admission.

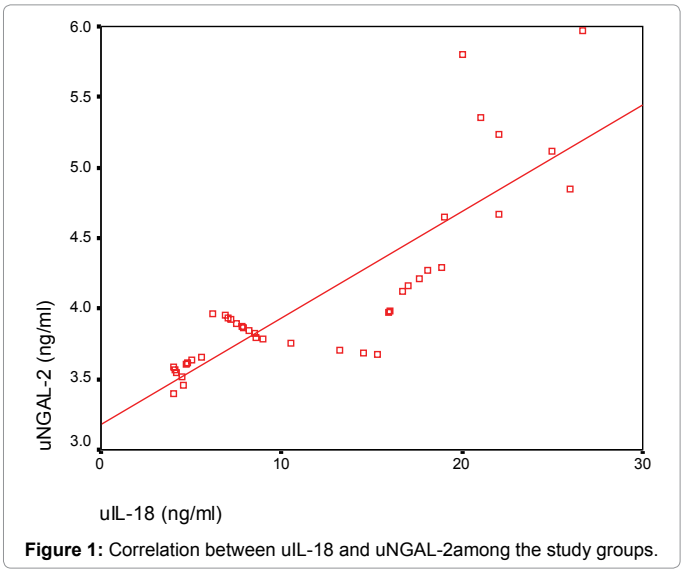

Figure 1: Correlation between uIL-18 and uNGAL-2among the study groups.

	uIL-18 (ng/ml)	uNGAL (ng/ml)	Creatinine (mg/dl)
Sensitivity (%)	95	92.5	41.52
Specificity (%)	82.5	80	52.69
Positive predictive value (PPV) (%)	84	82	66.3
Negative predictive value (NPV) (%)	94	91	70.3
Accuracy (%)	89	85	69.5
Cutoff	3.5	2.1	0.5

Table 5: This table shows sensitivity, specificity, predictive values and cutoff values of uNGAL, uIL-18 and serum creatinine.

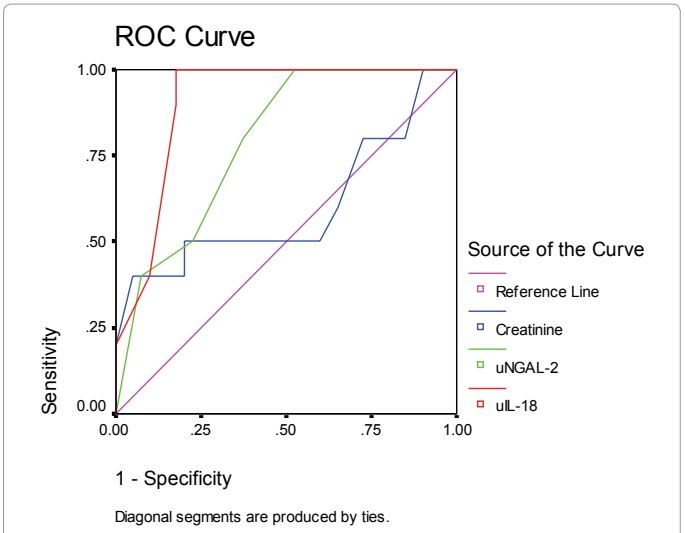

Figure 2: Receiver-operating curve (ROC) for uIL-18 and uNGAL-2 and serum creatinine.

Conclusion

Urinary NGAL and IL18 are excellent, simple and accurate markers in early diagnosis of renal injury in neonates which is earlier than serum creatinine.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all parents of the neonates included in the study.

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