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ORIGINAL ARTICLE

Analysis of urine biomarkers for early determination of acute kidney injury in non-septic and non-asphyxiated critically ill preterm neonates

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Abstract

Objective: We designed the present study to test the hypothesis that urinary biomarkers might predict acute kidney injury (AKI) development in non-septic and non-asphyxiated critically ill preterm infants. We evaluated urine (u) sistatin–C (uCys-C), kidney injury molecule–1 (uKIM–1) and neutrophil gelatinase associate lipocaline (uNGAL) as markers of AKI.
Methods: Sixty-four preterm infants with gestational age between 28 and 32 weeks were included in this study. Biomarkers were measured on day of life (DOL) 1, 3, and 7.
Results: uNGAL levels in the AKI group were significantly higher than in no-AKI group on DOL 1, 3 and 7 ($p = 0.016$, $p = 0.007$ and $p = 0.0014$, respectively).
Conclusions: uNGAL is sensitive, early, and noninvasive AKI biomarkers, increasing significantly in non-septic and non-asphyxiated critically ill preterm neonates.

Keywords

Acute kidney injury, kidney injury molecule–1, neutrophil gelatinase associate lipocaline, preterm infants, sistatin–C, urine

History

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Introduction

Acute kidney injury's (AKI) direct relation to lower hospital survival particularly in preterm neonates is gradually identified as a frequent and crucial neonatal morbidity [1–3]. Even though the actual incidence of AKI in preterm neonates is uncertain, the risk for this complication is significantly higher in more immature and ill neonates [2–4]. Previous studied revealed that the frequency of AKI in preterm neonates was reported in 12.5 and 18% [2,3]. The insufficient diagnostic performance of serum creatinine (SCr) may conclude to postponed diagnosis, in spite of the fact that high SCr levels are widely used in the identification of AKI [5]. For this reason, recent studies have concentrate on the clinical usefulness of obvious inventive AKI indicators such as cystatin C (Cys-C), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1), which is already assessed in different clinical situations of adults and children.

Cys-C discharged by all nucleated cells is a cationic cysteine protease inhibitor. It is known that Cys-C is unlimitedly filtered by the glomerulus and fully reabsorbed at the proximal tubules [6]. Studies have indicated decreased glomerular filtration rate (GFR) is linked to raised serum Cys-C (sCys-C) levels [6], and sCys-C is a better [7,8] indicator than sCre in identifying AKI. Besides, other studies illustrated urine cystatin C (uCys-C) levels are higher in AKI patients than individual controls in differing clinical situations, such as critical illness [9]. Urinary KIM-1 (uKIM-1) is extremely up-regulated in the proximal tubule epithelial cells, subsequent to hypoxic-ischemic and nephrotoxic injury, is a transmembrane protein. Therefore, it plays a role in tubulointerstitial damage [10]. After ischemic or toxic injury, tubular KIM-1 release was detected both in human and animal clinical studies [11,12]. Proteolytic separation of the extracellular territory of the protein causes to increased urine KIM-1 (uKIM-1) levels in AKI. uKIM-1 was considered to be extremely linked with ischemic acute tubular necrosis and useful in the diagnosis of AKI [13]. Neutrophil gelatinase-associated lipocalin (NGAL) owns to the lipocalin superfamily, secreted by different types of human tissues such as the kidney, is a 25-kD protein. After experimental ischemic or nephrotoxic injury, NGAL is one of the most strongly caused proteins by the kidney [14]. Studies have pointed out raised NGAL in the urine (uNGAL) and/or serum NGAL (sNGAL) of adults and children, emerging AKI compared to controls in miscellaneous clinical situations such as critical illness [15] was an early and sensitive biomarker. In addition, it is pointed out that NGAL has the potential to foresee AKI substantially more earlier than sCre [15,16]. As well as other important endpoints including mortality, the value of NGAL in diagnosing and anticipating AKI has currently been illustrated in critically ill preterm [17] and term neonates [18,19]. As a result, these indicators were found to be of important clinical worth in predicting AKI [7]. A very few

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studies have assessed the provisional connection of the AKI biomarkers in the neonatal period with regard to the beginning of kidney injury, as expressed by sCre considering to be the gold standard. Despite the fact that renal morbidity could not be eliminated, studies showed more immature neonates had higher concentrations of these markers in the urine [20] linked to continuing renal progress. Also, studies showed that it was demonstrated an important association between these biomarkers and asphyxiated neonates, sepsis, and urinary tract infection (UTI) in preterm neonates [19,21–27]. Therefore, we designed the present study to test the hypothesis that urinary biomarkers might predict AKI development in non-septic and non-asphyxiated critically ill preterm infants. To test this hypothesis, we compared uNGAL, uCys-C, and uKIM-1 in non-septic and non-asphyxiated critically ill preterm neonates with AKI (defined by elevated sCre) and healthy controls without AKI.

Material and patients

Patients and eligibility

This is a prospective case-control study of uNGAL, uCys-C, and uKIM-1 in non-septic and non-asphyxiated critically ill preterm infants between 28 and 32 gestational weeks (GW) who were admitted to the neonatal intensive care units (NICU) during the period of November 2011 and April 2013. Enrolled neonates were prospectively followed for AKI development during the first 7 postnatal days by measuring sCre at least once every other day. Any infants with clinically early-onset or culture-proven sepsis, acute perinatal asphyxia, received exchange transfusion; congenital heart disease except for PDA, renal or any other congenital chromosomal anomalies were excluded from the study. This study was totally enrolled 71 preterm infants. Of the 69 preterm neonates who met the inclusion criteria 3 infants for clinically early-onset sepsis and 2 infants with acute perinatal asphyxia and 2 infant for died was excluded from the study (Figure 1). The procedures were performed according to the ethical standard for human experimentations established by the Declaration of Helsinki of 1975, revised in 1983. The study was approved by the Ethical Committee of Inonu University and written consent forms were signed by the parents before the study.

Group categories and estimation of AKI

Seventy-four non-septic and non-asphyxiated critically ill preterm infants were enrolled in this prospective case-control study. They were stratified into two groups according to AKI status during the hospitalization. Clinical and laboratory characteristics of the AKI group were compared to non-AKI group.

Maternal data collected including age, gravidity, parity, pregnancy number, multiple pregnancies and marrying a relative. All infants were evaluated for possible prenatal risk factors for AKI (including pre-eclampsia/eclampsia, hypertension, maternal diabetes mellitus, gestational diabetes, premature rupture of membrane, maternal urinary tract infection, placenta previa, abruptio placenta, clinical chorioamnionitis, and maternal kidney dysfunction), and prenatal drug exposure.

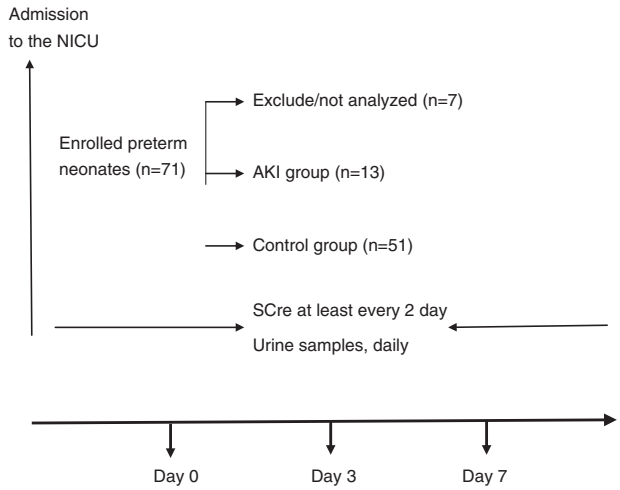

Figure 1. Study design showing the selection of cases and controls as well as the time points of the study in which serum creatinine (sCre) and urine neutrophil gelatinase-associated lipocalin (uNGAL), sistatin–C (uCys), and kidney injury molecule–1 (uKIM–1) were evaluated. NICU: neonate intensivecare unit, AK:I acute kidney injury, Scre: serum creatinine.

Demographic and perinatal characteristics including gestational age (GA), gender, birth weight (BW), height, small for gestational age (SGA), status, mode of delivery, intubation at birth, resuscutitation, and Apgar scores at 1 and 5 min, systolic and diastolic blood pressure were recorded. Additional parameters such as blood gases values at the admission, use of mechanical ventilation and continuous positive airway pressure (CPAP) treatment, use of postnatal steroids, use of surfactant, umbilical line (arterial or venous), and oliguria (urine output <0.5 ml/kg per hour for more than 8 h after birth) were also recorded. Urine output through bag or catheter collection was measured by 8 h intervals. All infants were also evaluated for the presence of respiratory distress syndrome (RDS), suspected or culture proven sepsis, severe perinatal asphyxia, pneumothorax, intra-ventricular hemorrhage (IVH), patent ductus arteriosus (PDA), hyperbilirubinemia, and the type of any treatment. GA was determined by calculation and/or antenatal ultrasonography and was confirmed by Ballard score postnatally. RDS was diagnosed according to clinical and radiographic criteria. The diagnosis of clinically sepsis was made by the Tollner scoring system. In addition, proven sepsis was defined as clinical suspicion of infection with a positive blood culture. Clinical sepsis was defined as a clinical suspicion of infection (at least 1 symptom from at least three categories), elevated serum CRP and increased immature/total neutrophils (I/T) ratio without a positive blood culture [28].

Premature rupture of membranes was defined as membrane rupture before the onset of the labor. Acute perinatal asphyxia was defined as: (i) profound metabolic or mixed acidemia (pH <7.00) in umbilical artery blood sample, if obtained, (ii) persistence of an Apgar score of 0–3 for longer than 5 min, (iii) neonatal neurologic squeal (e.g., seizures, coma, hypotonia), and (iv) multiple organ involvement (e.g., kidney, lungs, liver, heart, intestines) [29]. Results of head ultrasounds, chest X-rays, echocardiograms, and renal ultrasounds were also recorded.

Diagnosis of AKI

Diagnosis of AKI was defined as (a) SCr >1.5 mg/dl on postnatal (PN) Day 1 and sustained at least 48 h, while the mother has normal renal function. (b) Urine output <0.5 ml/kg/hour for 8 h and/or rising SCr values >0.3 mg/dl or more from baseline within 24 h [5]. Estimated glomerular filtration rate (GFR) (ml/min/1.73m^2) was calculated on PN Days 1 and 7 by using Schwartz Formula which is: k × height/SCr. The constant k was 0.33 for the infants born before 34 weeks of gestation. Fractional sodium excretion rate (FENa) levels were calculated with the equation of (urinary sodium/plasma creatinine) × 100/(plasma sodium/urinary creatinine).

Sampling measurements

Blood and urine samples were taken for BUN, SCr and uNGAL on PN Days 1, 3 and 7. Biochemical tests were performed at the same day except uNGAL, uCys, and uKIM-1 measurements.

Measurement of urinary concentrations of NGAL, Cys and KIM-1

The urine sample was centrifuged at 4000 rpm for 8 min and supernatant was stored at −70 °C until uNGAL measurement according to the manufacturer's protocol. The uNGAL and uCys levels were tested using Human Lipocalin-2/NGAL enzyme-linked immune sorbent assay (ELISA) kit and Human Cystatin C ELISA kit (BioVendor Research and Diagnostic Products, Asheville, NC) by ELISA method. KIM-1 concentrations in the urine were measured by a commercially available Human KIM-1 ELISA kit (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China). The enzymatic reactions were quantified in an automatic micro-plate photometer. The samples with higher concentrations were diluted and measured in duplicate. All intra-assay coefficients of variation were <10%. Creatinine levels were measured by the Kinetic Colorimetrik Jaffe method (Roche Diagnostics GmbH, Mannheim, Germany) with Cobas 8000 (Roche) autoanalyzer and levels were expressed as milligrams per deciliter. uCys-C, uNGAL, and uKIM-1 were standardized for changes in urine concentration using urine creatinine. Thus, urine concentrations hereafter referred to simply as uCys-C, uNGAL, and uKIM-1, are standardized unless otherwise stated (absolute urine values). All standardized values are expressed as ng/mg urine creatinine and absolute values in ng/ml (uCys-C, uNGAL and uKIM-1).

Statistical analysis

The statistical analysis of this study was performed using the Statistical Package for Social Sciences program (SPSS) for Windows version 16.0 (SPSS Inc., Chicago, IL). As a first step, normal distribution of the sample was analyzed by Shapiro–Wilk test. Normal distribution was represented by mean and standard deviation (SD), whereas skewed distribution was expressed by median (minimum–maximum). Unpaired t test or Mann–Whitney U test was used for comparison of the two groups. Categorical variables in proportions or percentages were analyzed at by Chi-square test or Fisher's exact test when appropriate. The relationship between uNGAL and other independent continuous variables was assessed by multiple linear regression analysis. Multivariate logistic regression analysis was used to determine whether an uNGAL levels was a predictor of AKI development on PN Day 1, independent of potential confounders. The diagnostic accuracy of the uNGAL levels on PN Day 1, 3 and PN Day 7 was tested for identifying AKI by constructing receiver operating characteristics (ROC) curves. Sensitivity and specificity of uNGAL were also calculated. A p value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

The study group was consisted of 13 preterm infants with AKI (AKI group) and 51 healthy preterm infants without AKI as the control group (non-AKI group). AKI has disappeared after the first week of life and there was no mortality in our study population till end of the study.

Preterm infants with AKI lower GA than the without AKI but this decrease is not statistically significant ($p = 0.071$). Compared to controls, preterm infants with AKI had significantly lower BW and birth length ($p = 0.019$ and $p = 0.001$, respectively). Preterm infants with AKI had significantly lower Apgar scores at 1 and 5 min, systolic and diastolic blood pressure (BP) and pH upon admission NICU ($p = 0.002$, $p = 0.0001$, $p = 0.0001$, $p = 0.0001$, and $p = 0.02$, respectively) (Table 1). In addition, intubation at birth, mechanical ventilator treatment, PDA, perinatal asfixia, IVH, and use of non-steroidal anti-inflammatory drugs (ibuprofen) were significantly different between AKI and non-AKI group ($p = 0.023$, $p = 0.027$, $p = 0.030$, $p = 0.030$, and $p = 0.030$, respectively). SGA, gender, mode of delivery, RDS, sepsis, meconium aspiration syndrome (MAS), surfactant treatment, use of umbilical lines (arterial or venous), pneumothorax, hyperbilirubinemia and continuous positive airway pressure (CPAP) treatment were not significantly different between the groups ($p > 0.05$, for each). There was no significant difference in the maternal characteristics between AKI and non-AKI group ($p > 0.05$, for each) except for abruptio plaencta ($p = 0.039$) (Table 2).

BUN, SCr and estimated GFR levels

Mean BUN and SCr levels in AKI group were similar to preterm infants without AKI on PN Day 1, and these results were not significantly different ($p > 0.05$, for each). Moreover, estimated GFR levels in AKI group were lower than preterm infants without AKI on PN Day 1 but the results were also not significantly different ($p > 0.05$). On the other hand, mean BUN and SCr levels were significantly higher in AKI group compared to preterm infants without AKI on PN Day 3 and 7 ($p = 0.002$, $p = 0.001$, $p = 0.0001$ and $p = 0.0001$, respectively). In addition, SCr-based estimated GFR levels in AKI group were significantly lower than control group on PN Day 3 and 7 ($p = 0.0001$, for each) (Table 3).

The urinary concentrations of NGAL

Median uNGAL levels were 10.0 (0.52–10.0) in non-AKI group versus 1.47 (0.15–10) in AKI group on PN Day 1. On PN Day 3, the levels were increased to 10 (0.63–10) in

Table 1. Clinical parameters of the preterm infants with and without AKI.

Parameter	AKI ($n=13$)	Non-AKI ($n=51$)	$p^{a,b}$
Gestational age (weeks)	29.1 ± 1.3	29.9 ± 1.4	0.071
Birth weight (g)	1145.9 ± 304.8	1396.0 ± 340.6	0.019
Birth length (cm)	35.2 ± 3.6	39.2 ± 3.7	0.001
Sex (M/F) (*n*)	7/6	24/27	0.761
SGA (*n*, %)	6 (46)	11 (21)	0.089
Vaginal delivery (*n*, %)	-	2 (3)	0.632
Apgar at 1 min	3 (3–5)	5 (3–5)	0.002
Apgar at 5 min	3 (3–7)	5 (3–7)	0.0001
pH upon admission	7.23 ± 0.1	7.30 ± 0.08	0.02
HCO_3	15.5 ± 4.6	15.5 ± 4.6	0.058
Base excise	-11.2 ± 5.3	-7.5 ± 3.9	0.006
Systolic BP (mmHg)	50 (50–65)	65 (60–80)	0.0001
Diastolic BP (mmHg)	30 (20–30)	30 (30–40)	0.0001
RDS (*n*, %)	7 (53.8)	18 (35.3)	0.340
Umbilical lines (*n*, %)	10 (76.9)	22 (43.1)	0.060
PDA (*n*, %)	7 (53.8)	10 (19.6)	0.030
Perinatal asfixia (*n*, %)	2 (15.4)	0 (0)	0.039
İVH (*n*, %)	2 (15.4)	0 (0)	0.039
Hyperbilirubinemia (*n*, %)	13 (100.0)	44 (%86.3)	0.328
MAS (*n*, %)	1 (7.6)	-	0.203
Sepsis (*n*, %)	2 (15.4).	1 (2.0)	0.102
NSAID (ibuprofen) (*n*, %)	7 (53.8)	10 (19.6)	0.030
Intubation at birth (*n*, %)	9 (69.2)	16 (31.4)	0.023
Resuscitation (*n*, %)	8 (61.5)	17 (33.3)	0.109
Mechanical ventilation (*n*, %)	9 (69.2)	17 (33.3)	0.027
CPAP (*n*, %)	13 (100)	49 (96.1)	0.632
Surfactant treatment (*n*, %)	7 (53.8)	18 (35.3)	0.340

AKI: acute kidney injury, M: Male, F: Female, SGA: small for gestational age, BP: blood pressure, RDS: respiratory distress syndrome, PDA: patent ducts arteriosus, IVH: intra-ventricular hemorrhage, MAS: mekonium aspiration syndrome, CPAP: continuous positive airway pressure, NSAID: non-steroidal anti-inflammatory drugs.

Data were presented as mean ± SD or median (minimum-maximum) or proportion and percentage. *p* values is for comparison between control and patients.

[a]Unpaired *t* test or Mann–Whitney U test.

[b]Chi-square test or Fisher's exact test, $p<0.05$ is significant.

Table 2. Maternal characteristics of the preterm infants with and without AKI.

Parameter	AKI ($n=13$)	Non-AKI ($n=51$)	$p^{a,b}$
Maternal age (years)	28.0 ± 6.1	30.7 ± 5.0	0.095
Pregnancy number	1.4 ± 0.7	2.2 ± 1.4	0.093
Multiple pregnancies (*n*, %)	7 (53.8)	16 (31.4)	0.195
Marrying a relative (*n*, %)	0 (0)	1 (2)	0.797
Prenatal steroids (*n*, %)	1 (7.7)	9 (17.6)	0.672
Preeclampsia/eclampsia (*n*, %)	1 (7.7)	14 (27.5)	0.269
Hypertension (*n*, %)	1 (7.7)	14 (27.5)	0.269
Diabetes mellitus (*n*, %)	1 (7.7)	5 (9.8)	0.648
Gestational diabetes (*n*, %)	0 (0)	2 (3.9)	0.632
PRM (*n*, %)	1 (7.7)	6 (11.8)	0.563
Maternal UTI (*n*, %)	–	–	–
Plasenta previa (*n*, %)	0 (0)	1 (2.0)	0.797
Abruptio plasenta (*n*, %)	2 (15.4)	0 (0)	0.039
Clinical chorioamnionitis (*n*, %)	1 (7.7)	1 (2.0)	0.368

PMR: Premature rupture of membrane, UTI: urinary tract infection.

Data were presented as mean ± SD or median (minimum-maximum) or proportion and percentage. *p* values is for comparison between control and patients.

[a]Unpaired *t* test or Mann–Whitney U test.

[b]Chi-square test or Fisher's exact test, $p<0.05$ is significant.

non-AKI group and 2.60 (0.15–10) in AKI group. On PN Day 7, the levels were decreased to 3.22 (3.0–10) in non-AKI group and were increased 2.93 (0.15–10) in AKI group. These results were significantly different in both on PN Day 1, 3 and PN day 7 ($p=0.016$, $p=0.007$ and $p=0.014$, respectively). On the other hand, there were no significant difference in the uKIM-1 and uCys levels between AKI and non-AKI group both PN Day 1, 3 and 7 ($p>0.05$, for each).

As uNGAL levels are relatively higher in premature infants likely due to renal immaturity [17], we performed a multiple linear regression analysis to control for potential confounders such as GA, BW, and gender. These factors were not significantly associated with uNGAL levels on the PN Day 1 and 7 ($p>0.05$, for each).

Backward stepwise logistic regression analysis identified that 5-min Apgar score (odd ratio (OR); 0.43, [95%CI; 0.23–0.813], $p=0.009$) and uNGAL levels (OR; 1.21; [95%CI; 1.01–1.44], $p=0.036$) were significantly associated with the development of AKI. Other confounders such as GA, BW, gender, and 1-min Apgar score was not significantly associated with the development of AKI ($p>0.05$, for each).

The level of uNGAL was predictive of AKI and the area under ROC curve was 0.71 for uNGAL levels for detecting of the development of AKI within 24 h ($p=0.017$) (Figure 2). In addition, calculated cutoff value for uNGAL levels to predict the development of AKI within 24 h was ≥38.3 ng/ml, with the sensitivity 66.7% and specificity 95.5% on PN Day 1. The positive and negative likelihood ratios were 14.8 and 1.02. On PN Day 7, the area under the ROC curve was obtained to be 0.78 for uNGAL levels detecting the development of AKI ($p=0.025$). At the concentration of uNGAL ≥ 30.6 ng/ml, the sensitivity and specificity for detecting AKI on PN Days 7 were 50% and 91%, and the positive and negative likelihood ratios were 5.4 and 1.04, respectively.

Discussion

We compared urine candidate AKI biomarkers in non-septic, non-asphyxiated critically ill preterm neonates with and without AKI (as it was defined by sCre) to those in healthy controls. According to our results, non-septic and non-asphyxiated critically ill preterm neonates had significantly increased levels of uNGAL on PN day 1, 3 and 7, suggesting of glomerular dysfunction. In contrast, uCyc and uKIM-1 levels were no significant difference in critically ill preterm neonates with and without AKI on PN day 1, 3 and 7. Our results have demonstrated that AKI is characterized by high levels of uNGAL. Therefore, uNGAL levels could be useful to predict subsequent development of AKI in critically ill preterm neonates.

Several potential risk factors for the development of AKI in preterm infants have been described [2,3,5]. In comparison to term infants, preterm infants are at higher risk of AKI because of prenatal fetal distress and exposure to multiple risk factors such as RDS, PDA, sepsis, intrauterine growth retardation, placental insufficiency, and maternal medications. In addition, the postnatal course of preterm infants is often complicated by the need for cardio–respiratory support, hypotension, and hypoxia [2,3,5]. Due to an incomplete nephrogenesis and lower number of nephrons, prematurity is

Table 3. Comparison of laboratory parameters of the preterm infants with and without AKI on postnatal day 1, 3 and 7.

Parameter	PND	AKI ($n=13$)	Non-AKI ($n=51$)	$p^{a,b}$
BUN (mg/dl)	1	12.8 ± 5.9	12.7 ± 5.2	0.943
	3	24.0 ± 16.4	13.8 ± 7.7	0.002
	7	22.7 ± 18.4	10.2 ± 6.6	0.001
SCr (mg/dl)	1	0.65 ± 0.1	0.65 ± 0.1	0.974
	3	0.98 ± 0.4	0.62 ± 0.09	0.0001
	7	1.0 ± 0.4	0.57 ± 0.1	0.0001
Uric acid	1	6.7 ± 2.5	5.8 ± 2.0	0.216
	3	7.9 ± 6.9	3.3 ± 1.8	0.0001
	7	6.1 ± 4.4	2.5 ± 0.8	0.0001
eGFR (ml/min/1.73m^2)	1	18.6 ± 4.4	20.9 ± 5.1	0.149
	3	14.0 ± 6.8	21.0 ± 4.6	0.0001
	7	13.8 ± 6.1	23.2 ± 5.3	0.0001
FENa (%)	1	0.79 (0.16–5.3)	1.6 (0.26–4.4)	0.931
	3	1.16 (0.60–5.28)	2.21 (0.29–31.2)	0.727
	7	1.92 (0.37–4.49)	1.42 (0.55–6.10)	0.495
Absolute urine values				
uNGAL (ng/ml)	1	10 (0.52–10)	1.47 (0.15–10)	0.016
	3	10 (0.63–10)	2.60 (0.15–10)	0.007
	7	3.22 (3.0–10)	2.93 (0.15–10)	0.014
uCysC (ng/ml)	1	87.85 (71.02–102.97)	84.60 (2.00–105.81)	0.468
	3	86.77 (75.95–98.80)	86.05 (58.75–110.89)	0.823
	7	90.14 (66.69–106.32)	87.13(65.97–132.77)	0.325
uKIM-1 (ng/ml)	1	1.21 (0.50–1.84)	0.77 (0.20–1.54)	0.198
	3	0.71 (0.43–1.67)	0.96 (0.51–1.70)	0.197
	7	0.69 (0.38–1.74)	1.14 (0.41–1.69)	0.284
Standardized urine values				
uNGAL (ng/mg uCre)	1	0.609 (0.028–1.62)	0.116 (0.009–2.0)	0.035
	3	0.462 (0.041–1.04)	0.183 (0.013–2.0)	0.025
	7	0.456 (0.094–2.0)	0.211 (0.009–2.0)	0.039
uCysC (ng/mg uCre)	1	8.23 (1.58–14.3)	6.89 (0.11–17.8)	0.775
	3	5.45 (1.33–13.9)	6.31 (2.58–16.4)	0.560
	7	5.11 (2.47–21.2)	6.69 (1.04–17.1)	0.646
uKIM-1 (ng/mg uCre)	1	0.062 (0.014–0.298)	0.071 (0.005–0.243)	0.511
	3	0.043 (0.014–0.266)	0.073 (0.019–0.242)	0.167
	7	0.054 (0.010–0.297)	0.069 (0.006–0.312)	0.448

PND: Postnatal days, AKI: Acute kidney injury, BUN: blood urea nitrogen, SCr: serum creatinine, Estimated GFR: Schwartz's estimated creatinine clearance, NGAL: urinary neutrophil gelatinase-associated lipocalin, uCysC: Urinary cystatin C, KIM-1: Urinary kidney injury molecule-1.

Data were presented as mean ± SD or median (minimum-maximum). p values is for comparison between control and patients.

[a]Unpaired t test or Mann–Whitney U test, $p<0.05$ is significant.

an independent risk factor for AKI by itself [30,31]. In addition, low GA, low BW, low Apgar score has been reported as an independent risk factor for impaired renal function in infants in the literature [2,3,5,30,31]. In our study, there was a significant difference between AKI and non-AKI preterm infants regarding BW, Apgar score, pH upon admission NİCU, systolic and diastolic BP, PDA, perinatal asfixia and İVH, mechanical ventilation treatment, NSAID (ibuprofen) use and intubation at birth, which is consistent with previous studies.

KIM-1 is a transmembrane type 1 epithelial cell protein playing a role in tubulointerstitial injury and being studied as a potential early biomarker of AKI in adults. A case–control study of 40 children undergoing cardiac surgery found increased uKIM-1 levels in the 20 patients with AKI [32]. Pre-operative investigation of the urinary biomarker profile of children with congenital hydronephrosis found that median uKIM-1/SCr levels were significantly greater in children with severe congenital hydronephrosis compared to controls [33]. uKIM-1 levels in newborns with AKI in the literature are limited and controversial. Gürkan et al showed that uKIM-1 levels can be used as a noninvasive biomarker of AKI and uKIM-1 levels can be an ideal biomarker in premature infants with RDS with AKI [34]. On the other hand, Sarafidis et al. [19] showed that uKIM-1 levels were comparable between asphyxiated neonates and healthy controls on DOL 1, 3 and 10 and did not discriminate the AKI group from the non-AKI group within the subgroup of asphyxiated neonates. Askenazi et al. demonstrated that uKIM-1 levels are higher in infants with AKI those of without AKI, but the differences were not statistically significant [35]. In another study by Askenazi et al. [17,20] showed that UKIM-1 levels were not statistically significant very low birth weight (VLBW) infants with and without AKI. But, maximum KIM-1 levels were higher in nonsurvivors than in survivors in this study. In our study, no significant differences in urine KIM–1 levels among the AKI and no-AKI groups were detected on postnatal days 1, 3 and 7. First option, this situation might be explained by the immaturity of the premature kidney. Another option is that preterm birth itself exposes the kidney to the risk of slight tubulointerstitial damage not manifesting with AKI, and most of the times goes undiagnosed. The decrease in uKIM-1 levels in these babies by time might suggest both hypotheses.

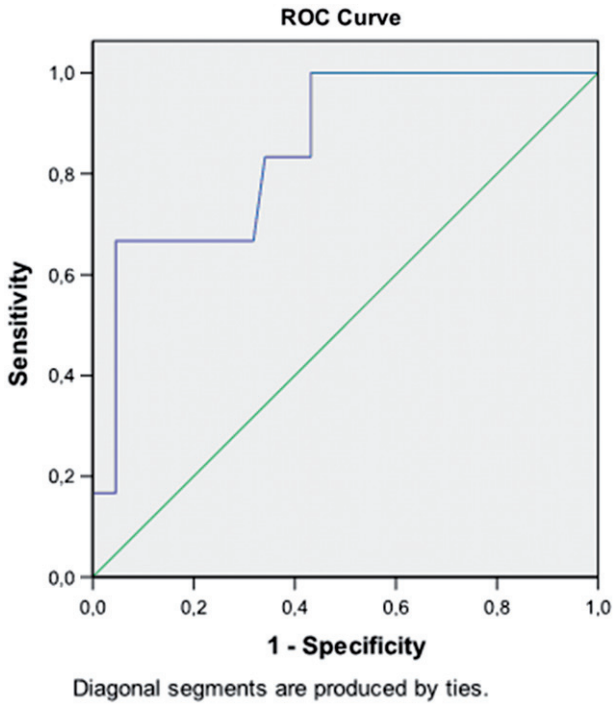

Figure 2. Receiver operating characteristic (ROC) curve analysis for uNGAL levels in predicting the development of AKI in non-septic and non-asphyxiated critically ill preterm infants on postnatal day 1 ($n = 50$). The level of uNGAL was predictive of AKI and the area under the curve (AUC) was obtained to be 0.85 for uNGAL levels detecting the development of AKI within 24 h ($p = 0.006$).

Urinary Cys-C concentration increases with renal tubular damage, independent of change in glomerular filtration rate [9]. uCys-C levels in newborns with AKI in the literature are limited and controversial. VLBW infant with AKI have higher maximum uCys-C concentrations during the first week of life than those without AKI, independent of GA and birth weight [20]. Some studies showed that uCys-C and sCys-C could predict AKI in newborns [17,19,20,35,36]. In another study by Askenazi et al. [17] showed that uCys-C levels were not statistically significant VLBW infants with and without AKI. In our study, we failed to document any significant value of uCys in the prediction of AKI on postnatal days 1, 3 and 7. This situation might be explained by the immaturity of the premature kidney. Another option is that preterm birth itself exposes the kidney to the risk of slight tubulointerstitial damage not manifesting with AKI, and most of the times goes undiagnosed. The decrease in uKIM-1 levels in these babies by time might suggest both hypotheses.

In many studies on children and adults NGAL rises significantly in patients with AKI but not in controls. Furthermore, this rise in NGAL occurs at 24 to 48 h before the rise in SCr is observed [27]. In a meta-analysis, which includes 19 studies and approximately 2500 patients, Haase et al. [37] reported a high diagnostic accuracy of NGAL with better predictive ability in children than in adults regarding diagnosis and prognosis of AKI. In a large study on AKI biomarkers in children undergoing cardiac surgery, Parikh et al. [38] found a significant association only between uNGAL levels and subsequent AKI development as well as poor outcomes. Of the candidate urine AKI biomarkers investigated in the current study, NGAL has been studied extensively in critically ill newborns with or without AKI. These studies suggested that NGAL may be a marker of systemic illness [21,22], and may have utility as an early sensitive screening marker for newborns at high risk for renal injury [23]. Askenazi et al. [17] reported that uNGAL could predict AKI and mortality in very preterm infants regardless of GA and BW. Sarafidis et al. [19] showed 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. Recent studies which are consistent to the previous studies, performing neonates, showed that uNGAL levels were higher in neonates with AKI compared with the neonates without AKI and suggested that uNGAL is a promising early biomarker of AKI in VLBW infants [19,21–27]. In our study, uNGAL concentration measured in the PN 1, 3 and 7 days of life were significantly higher in cases with AKI than cases without AKI, which is agreement with other studies conducted on neonates.

There are some limitations in the current study. First, the definition of AKI is based on the increase in SCr, although it remains as an accepted and widely used method for evaluating renal function in NICU. Second, because there is no clear and widely accepted definition of AKI in neonatal population, our definition criteria might show some discrepancy when our results are compared with the other studies. We could not measure SCr daily; therefore subjects may not have been enrolled in to the correct (AKI versus non-AKI) group. Therefore, exact timing of the kidney injury will remain unclear. This may restrict our ability to determine the exact time when the levels of uNGAL start to rise prior to SCr increases, and thus it limits our time-related diagnostic performance. Urinary creatinine levels must be controlled. We were unable to control for many known and unknown variables that could lead to both a rise in uNGAL and AKI.

Conclusions

Our results showed that uNGAL is associated with AKI development in non-septic and non-asphyxiated critically ill preterm infants. Our study supports ability of uNGAL for early detection of tubular dysfunction before GFR and SCr reaches to abnormal levels. uNGAL may reveal underlying AKI in critically ill preterm infants, and could be used as an early, sensitive, noninvasive and independent biomarker which predicts AKI development during early postnatal life. However, further studies with larger study groups are needed to better explore the role of uNGAL for the detection of AKI in the critically ill preterm infants with AKI.

Declaration of interest

The authors report no conflicts of interest.

References

1. Cataldi L, Leone R, Moretti U, et al. Potential risk factors for the development of acute renal failure in preterm newborn infants: a case – control study. Arch Dis Child Fetal Neonatal Ed 2005;90: F514–19.
2. Koralkar R, Ambalavanan N, Levitan EB, et al. Acute kidney injury reduces survival in very low birth weight infants. Pediatr Res 2011; 69:354–8.

3. Viswanathan S, Manyam B, Azhibekov T, Mhanna MJ. Risk factors associated with acute kidney injury in extremely low birth weight (ELBW) infants. Pediatr Nephrol 2012;27:303–11.
4. Walker MW, Clark RH, Spitzer AR. Elevation in plasma creatinine and renal failure in premature neonates without major anomalies: terminology, occurrence and factors associated with increased risk. J Perinatol 2011;31:199–205.
5. Jetton JG, Askenazi DJ. Update on acute kidney injury in the neonate. Curr Opin Pediatr 2012;24:191–6.
6. Bagshaw SM, Bellomo R. Cystatin C in acute kidney injury. Curr Opin Crit Care 2010;16:533–9.
7. Herget-Rosenthal S, Marggraf G, Hüsing J, et al. Early detection of acute renal failure by serum cystatin C. Kidney Int 2004;66: 1115–22.
8. Nejat M, Pickering JW, Walker RJ, Endre ZH. Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit. Nephrol Dial Transplant 2010;25:3283–9.
9. Herget-Rosenthal S, van Wijk JA, Bröcker-Preuss M, Bökenkamp A. Increased urinary cystatin C reflects structural and functional renal tubular impairment independent of glomerular filtration rate. Clin Biochem 2007;40:946–51.
10. Ichimura T, Hung CC, Yang SA, et al. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. Am J Physiol Renal Physiol 2004;286:F552–63.
11. Bonventre JV, Yang L. Kidney injury molecule-1. Curr Opin Crit Care 2010;16:556–61.
12. Ichimura T, Bonventre JV, Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998;273:4135–42.
13. Malyszko J, Bachorzewska-Gajewska H, Poniatowski B, et al. Urinary and serum biomarkers after cardiac catheterization in diabetic patients with stable angina and without severe chronic kidney disease. Ren Fail 2009;31:910–19.
14. Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinaseassociated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534–43.
15. Zappitelli M, Washburn KK, Arikan AA, et al. Urine neutrophil gelatinase associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. Crit Care 2007;11:R84.
16. Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005;365:1231–8.
17. Askenazi DJ, Montesanti A, Hunley H, et al. Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants. J Pediatr 2011;159:907–12.
18. Krawczeski CD, Woo JG, Wang Y, et al. Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass. J Pediatr 2011;158:1009–15.
19. Sarafidis K, Tsepkentzi E, Agakidou E, et al. Serum and urine acute kidney injury biomarkers in asphyxiated neonates. Pediatr Nephrol 2012;27:1575–82.
20. Askenazi DJ, Koralkar R, Levitan EB, et al. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. Pediatr Res 2011;70:302–6.
21. Parravicini E, Nemerofsky SL, Michelson KA, et al. Urinary neutrophil gelatinase-associated lipocalin is a promising biomarker for late-onset culture-positive sepsis in very low birth weight infants. Pediatr Res 2010;67:636–40.
22. Lavery AP, Meinzen-Derr JK, Anderson E, et al. Urinary NGAL in premature infants. Pediatr Res 2008;64:423–8.
23. Parravicini E. The clinical utility of urinary neutrophil gelatinase associated lipocalin in the neonatal ICU. Curr Opin Pediatr 2010; 22:146–50.
24. Sarafidis K, Tsepkentzi E, Diamanti E, et al. Urine neutrophil gelatinase-associated lipocalin to predict acute kidney injury in preterm neonates. A pilot study. Pediatr Nephrol 2014;29: 305–10.
25. Genc G, Avci B, Aygun C, et al. Urinary neutrophil gelatinase-associated lipocalin in septic preterm babies: a preliminary study. Am J Perinatol 2013;30:655–60.
26. Nejat M, Pickering JW, Walker RJ, et al. Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. Crit Care 2010;14:R85
27. Sweetman DU, Molloy EJ. Biomarkers of acute kidney injury in neonatal encephalopathy. Eur J Pediatr 2013;172:305–16.
28. Doellner H, Arntzen KJ, Haereid PE, et al. Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr 1998; 132:295–9.
29. Morales P, Bustamante D, Espina-Marchant P, et al. Pathophysiology of perinatal asphyxia: can we predict and improve individual outcomes? Epma J 2011;2:211–30.
30. Vásárhelyi B, Tóth-Heyn P, Treszl A, Tulassay T. Genetic polymorphisms and risk for acute renal failure in preterm neonates. Pediatr Nephrol 2005;20:132–5.
31. Rodríguez-Soriano J, Aguirre M, Oliveros R, Vallo A. Long-term renal follow-up of extremely low birth weight infants. Pediatr Nephrol 2005;20:579–84.
32. Han WK, Waikar SS, Johnson A, et al. Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int 2008;73: 863–9.
33. Wasilewska A, Taranta-Janusz K, Debek W, et al. KIM-1 and NGAL: new markers of obstructive nephropathy. Pediatr Nephrol 2011;26:579–86.
34. Genc G, Ozkaya O, Avci B, et al. Kidney injury molecule-1 as a promising biomarker for acute kidney injury in premature babies. Am J Perinatol 2013;30:245–52.
35. Elmas AT, Tabel Y, Elmas ON. Serum cystatin C predicts acute kidney injury in preterm neonates with respiratory distress syndrome. Pediatr Nephrol 2013;28:477–84.
36. Askenazi DJ, Koralkar R, Hundley HE, et al. Urine biomarkers predict acute kidney injury in newborns. J Pediatr 2012;161:270–5.
37. Haase M, Bellomo R, Devarajan P, et al. NGAL meta-analysis investigator group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009;54:1012–24.
38. Parikh CR, Devarajan P, Zappitelli M, et al. TRIBE-AKI consortium. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am Soc Nephrol 2011; 22:1737–47.