European Review for Medical and Pharmacological Sciences 2021; 25: 7078-7088

Biomarkers in neonates receiving potential nephrotoxic drugs

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Abstract. – OBJECTIVE: Novel biomarkers, such as kidney injury molecule-1 (KIM-1), cystatin, and neutrophil gelatinase-associated lipocalin (NGAL) were shown to predict acute kidney injury (AKI) earlier than serum creatinine in critically ill. We carried out the present study to evaluate these biomarkers in addition to conventional in our neonates.

PATIENTS AND METHODS: We recruited 70 neonates of various gestational age groups receiving one or more potential nephrotoxic drug/s. Daily urine samples were collected for estimating KIM-1, cystatin, and NGAL. Modified neonatal kidney disease improving global outcomes (mKDIGO) classification was used in defining AKI.

RESULTS: A significant trend in increased urine concentrations of KIM-1, cystatin, and NGAL were observed as we proceed from term to preterm categories. Strong positive correlation was observed between urine albumin and urine albumin creatinine ratio (ACR), and strong negative correlations between urine creatinine and urine cystatin, and between urine creatinine with urine NGAL. A moderate positive correlation was observed between urine KIM-1 and urine cystatin, between urine KIM-1 and urine NGAL, and between urine cystatin and urine NGAL; and a moderate negative correlation was observed between urine creatinine and urine KIM-1. Seven neonates met the mKDIGO criteria for AKI and ROC plot revealed that baseline KIM-1 and NGAL can significantly predict possible drug-induced AKI in neonates.

CONCLUSIONS: Urine KIM-1, cystatin, and NGAL are significantly correlated with several other conventional biomarkers that reflect renal function in critically ill neonates. Baseline urine KIM-1 and NGAL concentrations can predict the AKI following potential nephrotoxic drug use in this population.

Key Words:
KIM-1, Cystatin, NGAL, Gentamicin, Vancomycin, Furosemide.

Introduction

Kidneys undergo rapid changes during the early stages of life, especially in the neonatal period. Critically ill neonates are often administered drugs that mainly depend on the kidneys for elimination. Nephrotoxicity is the most common avoidable cause of acute kidney injury (AKI) in critically ill neonates and the most common entity associated with chronic kidney disease[1]. A recent report[2] indicated that 87% of neonates with AKI received one or more nephrotoxic drugs, among which furosemide (67.8%), vancomycin (28.7%) and gentamicin (21.4%) were the most frequently administered. The administration of nephrotoxic drugs is common in very low birth weight neonates, who receive approximately two weeks of nephrotoxic drugs or one drug every six days of hospitalisation[3]. A mortality rate between 50% and 80% was observed in neonates due to AKI[4].

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Several guidelines are used to diagnose AKI in neonates, namely the modified kidney disease improving global outcomes (mKDIGO) classification, modified AKI (mAKI), and paediatric risk, injury, failure, loss, end stage renal failure (pRIFLE)[5-7]. However, these guidelines are primarily based on elevations in serum creatinine and decreased urine output. Serum creatinine is influenced by several other factors, such as muscle mass, race, and catabolic states, including rhabdomyolysis and sepsis[8]. Additionally, 50% of kidney function must be affected for significant changes in serum creatinine, and a longer half-life of creatinine takes even more time to reflect the changes in serum and consequently to identify AKI[9]. Serum creatinine has been argued to be an inadequate gold standard for determining AKI[10]. Furthermore, false elevations of serum creatinine have been observed in conditions, such as hyperglycemia, hemolysis, and high total protein[11]. Hence, there is an ongoing search for other serum and urinary biomarkers for the early detection of AKI. Of these, urinary biomarkers in neonates are particularly interesting as they are non-invasively assessed. Secondly, there is a substantial limitation in the amount and number of blood samples that can be withdrawn, particularly from preterm neonates that can be overcomed with urine collection.

Urinary biomarkers, such as kidney injury molecule-1 (KIM-1), beta-2 microglobin (β2M), neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, clusterin osteopontin, osteoactivin, albumin and vascular endothelial growth factor, have been identified to be deranged in neonates with AKI[12]. The assessment of urinary biomarkers in the first four days of life has been shown to predict the likelihood of very low birth weight neonates developing AKI due to any cause[13]. Despite a good correlation between serum and urinary NGAL, only urinary NGAL predicted AKI better in neonates[14]. Cystatin C has been observed to predict AKI resulting from cardiopulmonary bypass in neonates[15]. Pre-clinical studies evaluating the renal toxicity of gentamicin revealed that clusterin, KIM-1 and osteopontin were elevated by 9.8-, 34.7- and 35.6-fold, respectively; biomarkers of glomerular damage and/or impairment of tubular reabsorption (CysC, β2M) increased 11.7- and 22.6-fold, respectively; and NGAL and α-GST increased <3-fold after two weeks of dosing[16]. Cystatin C, also known as post gamma globulin, is a non-glycosylated, 13.3-KDa protein belonging to the class of cystatin protease inhibitors. Cystatin C is normally catabolised in the proximal renal tubule and is not affected by gender, age, race, protein intake or muscle mass[17]. NGAL (a migration stimulating factor inhibitor) is a 25-KDa protein that covalently binds to matrix metalloproteinase-9 in the secondary granules of neutrophils[18]. KIM-1 is a type-I transmembrane protein belonging to the immunoglobulin superfamily and is not normally expressed in kidneys. The presence of KIM-1 in the urine is an indicator of proximal tubular injury[19]. A recent study[20] concluded that cystatin C, NGAL and KIM-1 in combination aid in the early detection of AKI in patients with liver cirrhosis. Considering the dearth of information on the utility of these biomarkers in the early detection of AKI in critically ill neonates receiving one or more of the drugs with nephrotoxic potential, we designed the present study.

Patients and Methods

Study Ethics and Design

We carried out a prospective observational study in the neonatal intensive care unit between September 2020 and April 2021 after obtaining approval from the Institutional Ethics Committee (E-06-PI-11/19) and the Ministry of Health (AURS/226/2020). We obtained written consent from either parent of the study participants.

Study Participants

We included neonates receiving one or more potential nephrotoxic drugs, such as gentamicin, vancomycin, furosemide, ibuprofen, or acetaminophen as a part of their standard of care. Those identified with major congenital abnormalities or renal disorders were excluded.

Study Procedure

Following consent by either parent of the eligible neonates, we collected demographic details (gestational age, birth weight, length, post-natal age, gender, diagnoses); Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) scores; drug-related details (name, dose, frequency, duration); laboratory parameters (serum creatinine, urine albumin, urine creatinine and urine spot albumin creatinine ratio); daily urine output; and daily body weight. We collected urine through either a urinary catheter (if already inserted as a standard of care) or, by placing either a urine bag or a sterile cotton wool ball placed inside the nappy daily until the potential nephrotoxic drug was stopped. The mKDIGO classification was used to

evaluate drug-induced nephrotoxicity, which was classified into the following stages: stage 1 – ≥ 26.53 µmol/L increase within 48 hours or ≥ 1.5-1.9 times from baseline and/or urine output ≤ 1 ml/kg/h for 24 hours; stage 2 – 2-2.9 times from baseline and/or ≤ 0.5 ml/kg/h for 24 hours; and stage 3 – ≥ 3 times baseline or serum creatinine ≥ 221.05 µmol/L or the initiation of renal replacement therapy and/or ≤ 0.3 ml/kg/h urine output for 24 hours[21]. The Naranjo algorithm was used to evaluate the causality of drug-induced nephrotoxicity[22]. Neonates were classified based on their gestational age as follows: extremely preterm (< 28 weeks); very preterm (28 to < 32 weeks); late preterm (32 to < 37 weeks); and term (≥ 37 weeks of gestation)[23]. Birth weights were classified as follows: ≥ 2.5 kg – normal; 1.5 to < 2.5 kg – low; 1 to < 1.5 kg – very low; and < 1 kg – extremely low. We followed Micromedex® NeoFax® Essentials 2020 dosing recommendations. The dosing regimen of the potential nephrotoxic drugs administered to the study population is described in Table I.

Estimations of Biomarkers

After collection, the urine samples were centrifuged for 10 min at 1500 x g, and the supernatant was stored in 0.5-ml aliquots at -80° C pending analysis. Enzyme-linked immunoassay (ELISA) kits from Quantikine® were used for the estimation of cystatin C, NGAL and KIM-1. Serum and urine creatinine were estimated *via* the enzymatic reaction method, which is based on the principles of Fossati, Prencipe and Berti. Urine albumin was estimated using a polyethylene glycol-enhanced turbidimetric assay. ADVIA® chemistry systems were used to estimate urine creatinine and albumin.

Statistical Analysis

Descriptive statistics were used to represent the demographic characteristics. The distributions of the numerical variables were checked for normal distribution using the Kolmogorov-Smirnov test, and Friedman's and Wilcoxon-signed rank-sum tests were used to compare fold and log 2-fold changes in the biomarkers during the nephrotoxic drug therapy. We compared the maximum fold and log 2-fold changes in the biomarkers between different nephrotoxic drugs using the Kruskal-Wallis H test. A Spearman's rank correlation test was used to evaluate the association between various biomarkers, bodyweight, and urine output. Spearman's rho (r_s) was considered weak (<0.4), moderate (0.4-0.59) and strong (≥0.6)[24]. Additionally, the percent variability in the biomarkers contributed by gestational age (as a categorical variable) was

Table I. Dosing regimen of potential nephrotoxic drugs in the study population.

Drugs	Post menstrual age (weeks)	Postnatal age (days)	Dose (mg/kg)	Dosing interval (hours)e
Amikacin	< 29	0 to 7	18	48
		8 to 28	15	36
		> 29	15	24
	30 to 34	0 to 7	18	36
		> 8	15	24
	> 35	All	15	24
Gentamicin	<29	0 to 7	5	48
		8 to 28	4	36
		>29	4	24
	30 to 34	0 to 7	4.5	36
		>8	4	24
	>35	All	4	24
Vancomycin	≤29	0 to 14	10	18
		> 14		12
	30 to 36	0 to 14		12
		> 14		8
	37 to 44	0 to 7		12
		> 7		8
	> 45	All		6
Furosemide	1 mg/kg/dose initial dose titrated to a maximum dose of 2 mg/kg/dose; administered every 24 hours in preterm and 12 hours in term neonates.			
Acetaminophen	15 mg/kg/dose every six hours.			
Ibuprofen	10 mg/kg bolus followed by 5 mg/kg every 24 hours.			

Table II. Summary of the baseline characteristics of the study participants (N= 70).

Variables		Values
Gestational age (weeks)		33.3 (20.1-39.8)
Gestational age categories (n)	Term	13
	Late preterm	33
	Very preterm	12
	Extremely preterm	12
Male: Female (n)		41: 29
Birthweight (kg)		1.62 (0.48-5.3)
Birthweight categories (n)	Normal	18
	Low	22
	Very low	19
	Extremely low	11
Length (cm)	41.5 (25-54)	
	First minute	8 (0-9)
	Fifth minute	9 (1-10)
	Tenth minute	10 (2-10)
Serum creatinine (µmol/L)		67.5 (39-110)
Urine creatinine (µmol/L)		813 (255-4762)
Urine albumin (mg/L)		19.5 (3-361)
Urine spot ACR (mg/mmol)		18.8 (3.8-760.8)
Urine KIM-1 (ng/mg urine creatinine)		0.85 (0.14-6.6)
Urine cystatin (ng/mg urine creatinine)		6.63 (0.5-32.9)
Urine NGAL (ng/mg urine creatinine)		38.45 (7.7-288.5)

All the values are stated in median (range) unless specified otherwise; ACR – Albumin creatinine ratio.

evaluated using the Eta (η) coefficient test. The η values represent the proportion of total variability of the evaluated biomarkers accounted for by the gestational age. The trend in urine KIM-1, cystatin C and NGAL across the gestational age groups was evaluated using the Jonckheere-Terpstra test, and the Kruskal-Wallis H test was used to assess the difference in the median values between the groups. A receiver operating characteristics (ROC) curve was generated for urine KIM-1, cystatin and NGAL to detect nephrotoxicity. Cut-off values were determined by the lowest distance to the top-left corner of the ROC curve as determined by the square root [(1-sensitivity)^2 + (1-specificity)^2]. Considering the wide range in the incidence of nephrotoxicity reported (between 10% and 80%) and the anecdotal evidence from in-house neonatologists indicating nephrotoxicity of approximately 20%, we estimated the sample size with a 95% confidence interval, at 80% power and with 5% precision to 70 neonates. A *p*-value of ≤ 0.05 was considered significant. SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0, Armonk, NY, USA) was used for statistical analysis.

Results

Demographic Characteristics

A majority of the study participants were late preterm neonates and had a low birth weight. A summary of the demographic characteristics, including the baseline values of the biomarkers, is given in Table II. Concomitant diagnoses were as follows: respiratory distress syndrome (n = 34), suspected neonatal sepsis (n = 14), congenital heart disease (n = 39), neonatal jaundice (n = 38), intraventricular haemorrhage (n = 17), pneumonia (n = 9), hypoxic ischemic encephalopathy (n = 8), disseminated intravascular coagulation (n = 7), retinopathy of prematurity (n = 5), meconium aspiration syndrome (n = 3) and necrotising enterocolitis (n = 2).

Correlation Between the Biomarkers

The scatterplot matrix (including the histogram) depicting the association between biomarkers, urine output and body weight is displayed in Figure 1. A strong positive correlation was observed between urine albumin and urine ACR, and between urine output and body weight. A strong

negative correlation was found between urine creatinine and urine cystatin, and between urine creatinine with urine NGAL (Table III). A moderate positive correlation was observed between urine KIM-1 and urine cystatin, urine KIM-1 and urine NGAL, and urine cystatin and urine NGAL. A moderate negative correlation was found between urine creatinine and urine KIM-1, urine ACR and body weight, and urine cystatin and body weight. Either a weak or no correlation was observed for the remaining biomarkers.

A significant trend in increased concentrations of urine KIM-1 ($p = 0.021$), cystatin ($p = 0.0001$) and NGAL ($p = 0.007$) was observed as we proceeded from term to various preterm categories (Figure 2). Extremely preterm neonates had significantly greater concentrations of urine KIM-1 ($p = 0.043$), cystatin ($p = 0.0001$) and NGAL ($p = 0.007$) compared to late preterm neonates. The term neonates had significantly lower urine cystatin concentrations compared to extremely preterm neonates ($p = 0.0001$). Analysis of the

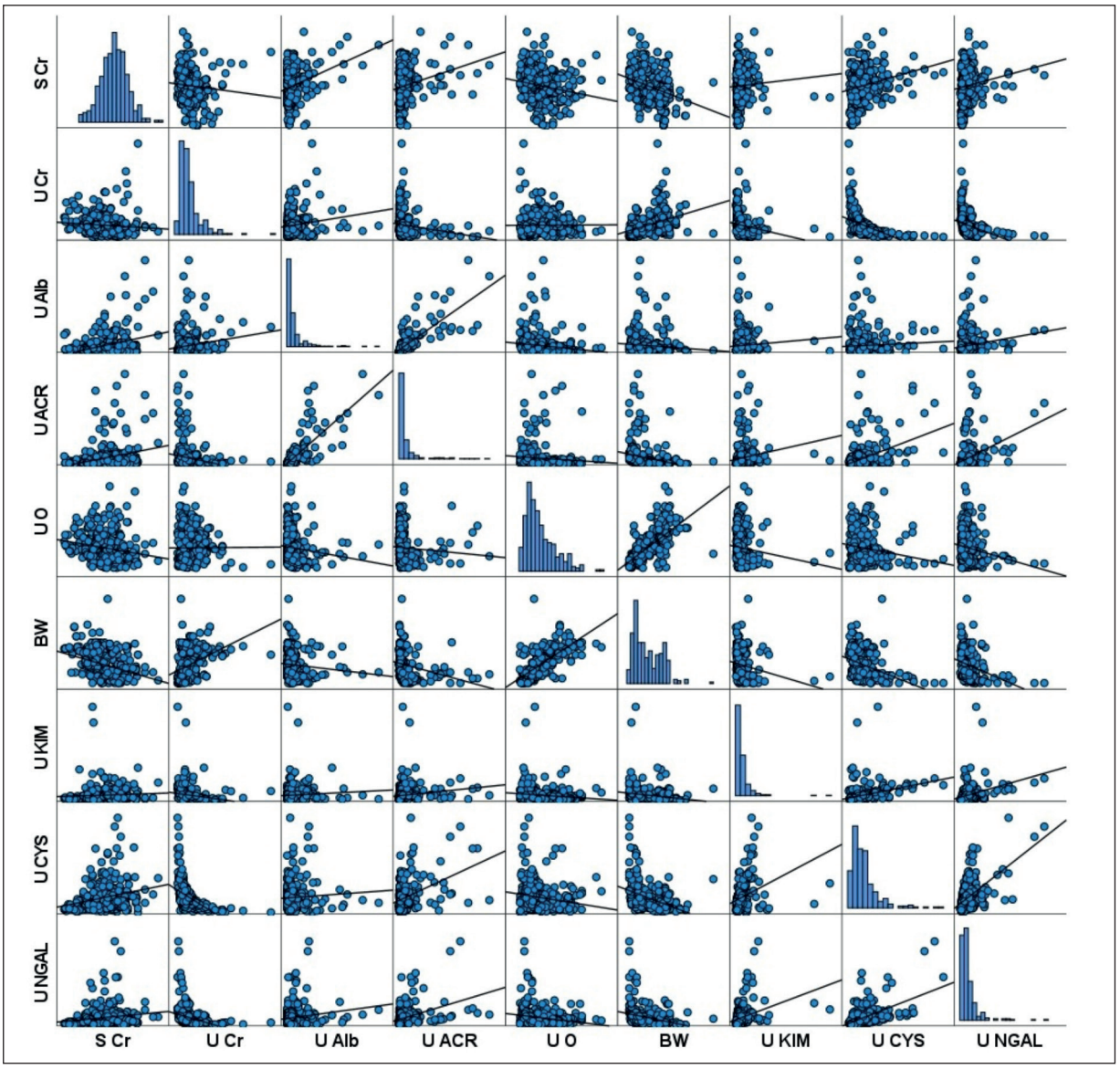

Figure 1. Correlation between biomarkers, urine output, and body weight. This scatterplot matrix describes the correlations between serum creatinine (S Cr), urine creatinine (U Cr), urine albumin (U Alb), urine ACR (U ACR), urine output (UO), body weight (BW), urine KIM-1 (U KIM), urine cystatin (U CYS), and urine NGAL (U NGAL). The histograms of the respective parameters are represented diagonally.

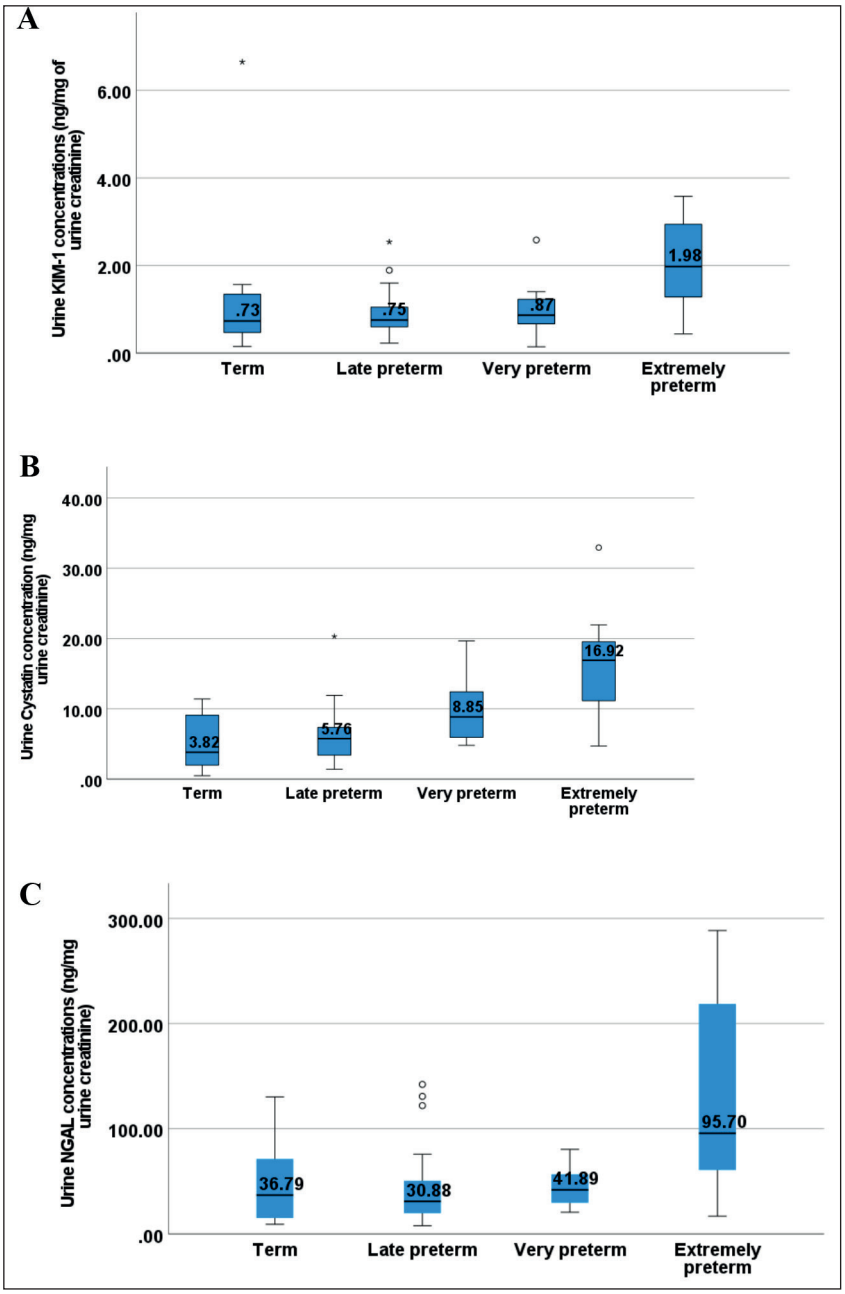

Figure 2. Baseline urine KIM-1, cystatin, and NGAL concentrations according to gestational age groups. The boxplots depict the values of urine KIM-1 **(A)**, cystatin **(B)**, and NGAL **(C)** in the study population according to the gestational age categories.

effect of gestational age categories on biomarkers revealed a moderate positive association with urine cystatin (r_s: 0.568; η: 0.573), a weak positive association with urine KIM-1 (r_s: 0.32; η: 0.227), urine NGAL (r_s: 0.346; η: 0.471), urine ACR (r_s: 0.349; η: 0.263), serum creatinine (r_s: 0.254; η: 0.267) and urine albumin (r_s: 0.13; η: 0.069), and a moderate negative association with urine creatinine (r_s: -0.45 ; η: 0.421).

Details of the Potential Nephrotoxic Medications

Sixty-seven neonates received gentamicin with the median (range) duration of five (1-9) days. Intravenous acetaminophen was administered to 14 neonates with the median (range) duration of four (1-9) days. Intravenous furosemide was administered to 27 neonates for three (1-16) days. Vancomycin was administered to three neonates with

Table III. Correlation co-efficient between biomarkers, urine output, and body weight.

Variables	Parameters	Serum Creatinine	Urine Creatinine	Urine Albumin	Urine ACR	Urine output	Body weight	Urine KIM-1	Urine Cystatin	Urine NGAL
Serum	rs	NA	-0.205*	0.148*	0.260*	-0.265*	-0.318*	0.168*	0.273*	0.276*
Creatinine	N	425	425	425	425	419	422	371	373	391
Urine	rs	-0.205*	NA	0.288*	-0.175*	0.113*	0.380*	-0.489*	-0.729*	-0.670*
Creatinine	N	425	431	431	431	425	428	377	378	397
Urine Albumin	rs	0.148*	0.288*	NA	0.867*	-0.285*	-0.224*	0.057	-0.033	0.053
	N	425	431	431	431	425	428	377	378	397
Urine ACR	rs	0.260*	-0.175*	0.867*	NA	-0.358*	-0.421*	0.282*	0.321*	0.387*
	N	425	431	431	431	425	428	377	378	397
Urine output	rs	-0.265*	0.113*	-0.285*	-0.358*	NA	0.759*	-0.222*	-0.248*	-0.201*
	N	419	425	425	425	425	422	371	372	391
Body weight	rs	-0.318*	0.380*	-0.224*	-0.421*	0.759*	NA	-0.320*	-0.469*	-0.396*
	N	422	428	428	428	422	428	375	375	395
Urine KIM-1	rs	0.168*	-0.489*	0.057	0.282*	-0.222*	-0.320*	NA	0.542*	0.509*
	N	371	377	377	377	371	375	377	339	368
Urine Cystatin	rs	0.273*	-0.729*	-0.033	0.321*	-0.248*	-0.469*	0.542*	NA	0.524*
	N	373	378	378	378	372	375	339	378	358
Urine NGAL	rs	0.276*	-0.670*	0.053	0.387*	-0.201*	-0.396*	0.509*	0.524*	NA
	N	391	397	397	397	391	395	368	358	397

*-Statistically significant; rs represents Spearman-rho; N – total number of samples analyzed; NA – Not applicable.

the median (range) duration of nine (1-11) days. Intravenous ibuprofen was administered to four neonates with the median (range) duration of 3.5 (2-4) days. Lastly, two neonates received amikacin, one for five days and the other for six days. During the study period, 15 neonates received gentamicin and furosemide, two received gentamicin, furosemide, and ibuprofen, two received gentamicin, furosemide, and vancomycin, two received gentamicin and ibuprofen, six received gentamicin and acetaminophen, four received gentamicin, acetaminophen, and furosemide, and one was administered acetaminophen with furosemide. One neonate initially received gentamicin along with acetaminophen and furosemide, but gentamicin was later replaced with amikacin, which was administered with acetaminophen and furosemide. Similarly, another neonate initially received gentamicin, which was later changed to vancomycin and then to amikacin, again co-administered with acetaminophen and furosemide.

Changes in the Biomarkers with Potential Nephrotoxic Drug Use

Fold changes and log 2-fold changes of serum creatinine and urine biomarkers are depicted in Electronic **Supplementary Figures 1-14**, which reveal no significant trends in any of the biomarkers. Similarly, no significant differences were observed in any of the biomarkers during the gentamicin therapy (Electronic **Supplementary Figures 15-28**), the gentamicin + acetaminophen therapy (Electronic **Supplementary Figures 29-42**), the gentamicin + furosemide therapy (Electronic **Supplementary Figures 43-56**), the gentamicin + ibuprofen therapy (Electronic **Supplementary Figures 57-70**), the acetaminophen + furosemide therapy (Electronic **Supplementary Figures 71-84**) or the furosemide therapy (Electronic **Supplementary Figures 85-98**). Due to number constraints, we could not evaluate the changes in the biomarkers for the neonates who received vancomycin, acetaminophen, and amikacin alone.

The maximum fold and log 2-fold changes in the biomarkers between different nephrotoxic drugs are depicted in Electronic **Supplementary Figures 99-112**, which reveal no significant differences between the groups.

Biomarkers in Neonates with AKI

Seven (10%) neonates met the criteria for AKI, and a possible causal association between nephrotoxic drugs and AKI was detected in all these neonates. Six of these neonates received gentamicin, and one received furosemide (Table IV). Three were diagnosed with changes in serum creatinine, whereas the others met the criteria for urine output. The ROC curve of baseline urine KIM-1, cystatin and NGAL in predicting

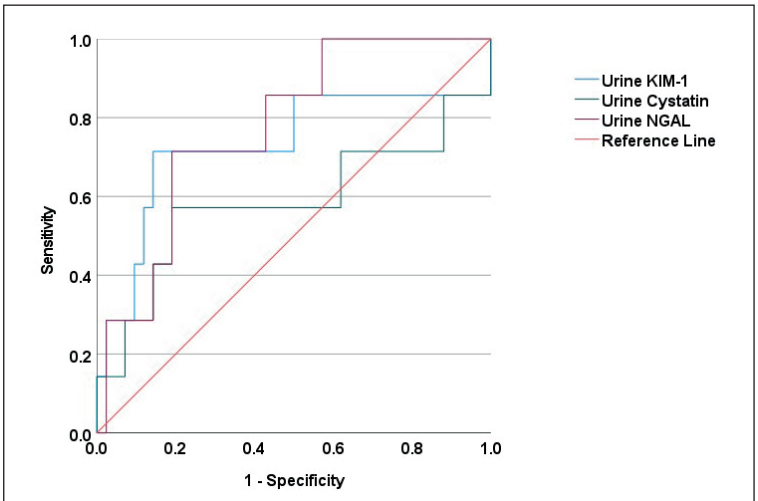

Figure 3. ROC curve of urine KIM-1, cystatin, and NGAL between neonates with AKI and others.

AKI showed a statistically significant area under the curve for NGAL (area: 0.776; 95% CI for the area: 0.61-0.94; $p = 0.021$) and KIM-1 (area: 0.731; 95% CI for the area: 0.475-0.987; $p = 0.05$) but not for cystatin (area: 0.585; 95% CI for the area: 0.3-0.874; $p = 0.5$) (Figure 3). The cut-off values for baseline KIM-1 and NGAL were 1.55 and 62.4 ng/mg urine creatinine, respectively, in determining potential drug-induced AKI.

Discussion

Key Findings from the Present Study

We carried out the present study to evaluate the utility of various biomarkers in 70 neonates receiving one or more potential nephrotoxic drugs, including gentamicin, acetaminophen, ibuprofen, furosemide, vancomycin, and amikacin. A significant trend in increased urine concentrations of KIM-1, cystatin and NGAL were observed as we proceeded from term to preterm categories. A strong positive correlation was observed between urine albumin and urine ACR, and a strong negative correlation was found between urine creatinine and urine cystatin, and between urine creatinine with urine NGAL. A moderate positive correlation was observed between urine KIM-1 and urine cystatin, urine KIM-1 and urine NGAL, and urine cystatin and urine NGAL. A moderate negative correlation was observed between urine creatinine and urine KIM-1. No significant differences in either fold or log 2-fold changes were observed in any of the evaluated biomarkers during potential nephrotoxic drug therapy. Seven neonates met the mKDIGO criteria for AKI, and the ROC plot revealed that baseline KIM-1 and NGAL can significantly predict AKI in neonates.

Comparison with Other Studies

Novel biomarkers, such as KIM-1, cystatin and NGAL, were shown to better predict and identify AKI in neonatal populations[25]. Although these biomarkers could be detected in both serum and urine, urinary levels are better predictors owing to the influence of systemic inflammation, which may derange the levels of these biomarkers in the blood[26]. We observed urine KIM-1 and NGAL but not cystatin to be a significant predictor of AKI. This is consistent with a study by Correa et al[27] in which no changes in urine cystatin were observed during the initial few days of life in contrast to KIM-1 and NGAL. Urine NGAL has been shown[28,29] to elevate 48 hours before creatinine in AKI in the Emergency Department, and due to sepsis. Despite several reports, the validation of KIM-1, cystatin and NGAL concentrations for establishing the normal reference ranges in the neonatal population has not yet occurred. This is a major obstacle, one which prevents guidelines related to AKI from providing criteria-based recommendations on the definition and severity states of AKI. The current versions of the guidelines defining AKI depend on serum creatinine

and urine output. Serum creatinine, in addition to taking a longer time to exhibit an increase in the blood regarding the onset of kidney injury, is also influenced by maternal levels of creatinine in the first few days of neonatal life[30]. However, KIM-1, cystatin and NGAL, due to their larger molecular weight, do not cross the placenta. As such, their levels in neonates during the first few days of life reflect only the renal synthesis from the neonates. A significant relationship between gestational age groups and urine KIM-1, cystatin and NGAL were observed in the present study. Our findings agree with a previous study[31] carried out exclusively amongst premature neonates. Hence, there is a need to conduct large population-based studies to elucidate the normal reference ranges and cut-off levels of various biomarkers in defining kidney injury. Considering the baseline differences between the gestational age categories, we evaluated the fold and log 2-fold changes of the biomarkers from the baseline.

Urine KIM-1, cystatin and NGAL concentrations are affected by the hydration status of individuals, and therefore, estimations from 24-hour samples are preferred[32]. However, as this is not practically feasible, spot urine sample estimations are often used. In these estimations, KIM-1, cystatin and NGAL concentrations are conventionally expressed per unit quantity of urine creatinine. KIM-1, cystatin and NGAL undergo extensive tubular re-absorption and are thus barely detectable in the urine[33]. However, creatinine, besides glomerular filtration, is also secreted by the tubules in the nephrons[34]. Hence, it is arguable whether the concentrations of these urinary biomarkers should be corrected with urine creatinine values. We observed negative correlations between urine KIM-1, cystatin and NGAL with urine creatinine. This is consistent with a recent study on an adult population in which the authors concluded that urine cystatin concentrations were negatively correlated with creatinine and should thus not be

Table IV. Neonates identified with AKI.

S. No.	Gestational age (weeks)	Potential nephrotoxic drug/s received	mKDIGO stage; Criteria for diagnosis of AKI.	Baseline serum creatinine (µmol/L) and urine output (ml/kg/h)	Fold and log 2-fold change in urine KIM-1; Baseline urine KIM-1 (ng/mg urine creatinine) at the time of AKI diagnosis	Fold and log 2-fold change in urine Cystatin; Baseline urine cystatin (ng/mg urine creatinine) at the time of AKI diagnosis	Fold and log 2-fold change in urine NGAL; Baseline urine NGAL (ng/mg urine creatinine) at the time of AKI diagnosis
1.	22.1	Gentamicin	1; Serum creatinine absolute change: 28 µmol/L, and 1.56 times elevation from baseline.	50; 4.3	1.7; 0.8; 2.16	2; 1; 19.2	2; 1; 223.6
2.	37	Gentamicin	1; Urine output: 0.6 ml/kg/h	70; 1.1	3; 1.6; 0.76	0.1; -3.8; 11	0.2; -2.1; 84.9
3.	37.7	Gentamicin	1; Urine output: 0.9 ml/kg/h	95; 1	0.3; -1.7; 6.6	0.2; -2.2; 2.6	0.3; -1.7; 63.5
4.	39.6	Gentamicin	1; Urine output: 0.9 ml/kg/h	73; 1	1.3; 0.4; 1.6	0.5; -0.9; 5.1	0.4; -1.3; 71.2
5.	27	Gentamicin	1; Urine output: 0.7 ml/kg/h	67; 5	1.4; 0.5; 1.8	1.3; 0.3; 13.6	1.3; 0.4; 32.1
6.	27.1	Gentamicin	1; Serum creatinine 1.5 times elevation from baseline	48; 4	0.2; -2.7; 3.5	0.2; -2.3; 21.94	0.6; -0.8; 255.7
7.	33	Furosemide	1; Serum creatinine 1.6 times elevation from baseline	54; 2.7	2.3; 1.2; 0.6	6.1; 2.6; 4.1	2.02; 1.01; 45.1

expressed per unit quantity of urine creatinine[35]. Another study[36] observed negative correlations between urine NGAL and creatinine in a subgroup of adults with chronic kidney disease and the possibility of overadjustment risk. However, there are no such reports from neonates. Furthermore, as there is no consensus on how to report the urine values of KIM-1, cystatin and NGAL, we recommend reporting both the absolute values and corrected values with urine creatinine until further evidence emerges.

Strengths and Weakness of the Present Study

The present study was exclusively carried out in neonates receiving various nephrotoxic drugs. However, we did not enrol neonates with more severe AKI stages to compare the utility of these biomarkers in identifying AKI severity. Despite achieving an optimal sample size for the overall study objective, it may be inadequately powered for evaluating the differences between the drug or drug combinations. This could be one reason why we were unable to observe any significant differences between the nephrotoxic drugs in the present study.

Conclusions

Urine KIM-1, cystatin and NGAL are significantly correlated with several other conventional biomarkers that reflect renal function in neonates. Baseline urine KIM-1 and NGAL concentrations can predict AKI following potential nephrotoxic drug use in this population.

Funding

The study was carried out with the intra-mural grant (G05/AGU-11/19) from College of Medicine & Medical Sciences, Arabian Gulf University.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgement

We thank the RTST, Ministry of Health, Kingdom of Bahrain for providing approval to carry out this study. We are also grateful to Dr. Mai Sater, Assistant Professor, Department of Medical Biochemistry, CMMS, AGU for providing access to use spectrophotometer.

Data Availability Statement

The data is available with the corresponding author and can be shared upon a reasonable request.

Ethics Approval and Consent

The study was approved by the Institutional Ethics Committee (E-06-PI-11/19), CMMS, Arabian Gulf University, and the Ministry of Health (AURS/226/2020). Written consent was obtained from either parent of each study participant.

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