IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

Open Access
Original Article

Accuracy of Urine Calprotectin in the Diagnosis of Acute Kidney Injury in Neonates: A Cross-Sectional Study

Nasrin Khalesi[1], Shima Mohammadian[2], Nakysa Hooman[3], Mahmoud Khodadost[4]*, Leila Allahqoli[5]

1. Department of Pediatrics, Ali Asghar Hospital, Iran University of Medical Sciences, Tehran, Iran
2. Aliasghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Department of Pediatric Nephrology, Aliasghar Children Research Development Center (ACRDC), Iran University of Medical Sciences, Tehran, Iran
4. Department of Epidemiology, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran & Department of Epidemiology, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
5. Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: Urine calprotectin significantly elevates in acute kidney injury (AKI) in adult and pediatric patients. The present study aimed to assess the accuracy of urine calprotectin as a diagnostic marker for (AKI) in neonates.

Methods: This cross-sectional study assessed urine calprotectin in 100 neonates (80 newborns with confirmed AKI and 20 healthy ones). Random urine calprotectin was measured by Enzyme-linked Immunosorbent Assay (ELISA) and then compared between the two groups. We included the neonates who had received at least 48 h of intravenous fluid and met the inclusion and exclusion criteria. Receiver-operating characteristic (ROC) curve was used to set a cut-off point for urine calprotectin for the prediction of AKI. The overall accuracy and Kappa coefficient were used to assess the agreement between the two methods. A p-value less than 0.05 was considered statistically significant.

Results: Urine calprotectin levels were not significantly higher in neonates with AKI, as compared to those in the healthy ones (146.2 versus 142.4; P=0.1). The results pointed to an optimal cut-off value of 123.5 mg/dl for urine calprotectin with the area under the curve of 0.515 (the sensitivity, specificity, positive predictive value, and negative predictive value were obtained at 77.5%, 40%, 83.7%, and 30.7%, respectively). The overall accuracy and Kappa agreement coefficient were reported as 70% and 0.15, r (P=0.11).

Conclusion: As evidenced by the results of the resent study, although urine calprotectin level elevates in AKI in neonates, it is not more sensitive than gold standards to predict AKI.

Keywords: Acute kidney injury, Neonate/infant, Plasma creatinine, Urine calprotectin

Introduction

Acute renal failure is a common cause of morbidity in neonates admitted to neonatal intensive care units (NICUs) (30% worldwide) (1). Mortality and lengths of hospital stay in neonates with acute kidney injury (AKI) are higher than those without AKI (2), and the risk of chronic renal failure in adulthood increased in pediatric patients with AKI (3). It is obvious that the prognosis of AKI is highly dependent on early diagnosis and onset of treatment. Therefore, it is of utmost importance to diagnose AKI with respect to physiologic changes in neonates. Nephrogenesis begins from the 5th week of gestational age and will be completed until adulthood (4). There is a paucity of studies on the effect of prematurity and intrauterine growth restriction (IUGR) on nephrogenesis; nonetheless, the adverse effect of renal failure on renal maturation is obvious (5, 6). Definition of AKI for neonates, in a neonatal AKI workshop which was held in 2017, was modified from pediatrics definition based on serum creatinine and urine output (7).

Neonatal serum creatinine levels (nSCr) could

* *Corresponding author*: Mahmoud Khodadost, Department of Epidemiology, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran & Department of Epidemiology, School of Public Health, Iran University of Medical Sciences, Tehran, Iran. Email: mahmodkhodadost@yahoo.com

Please cite this paper as:
Khalesi N, Mohammadian SH, Hooman N, Khodadost M, Allahqoli L. Accuracy of urine calprotectin in the diagnosis of acute kidney injury in neonates: a cross-sectional study. Iranian Journal of Neonatology. 2021 Oct: 12(4). DOI: 10.22038/IJN.2021.53102.1962

be affected by birth weight (BW) and maternal serum creatinine (mSCr) (8). Renal function is low at birth, especially in premature neonates. In addition, preterm birth is associated with maternal complications. Therefore, nSCr in preterm neonates is influenced by various factors (8, 9). Serum creatinine in neonates could be affected by pregnancy-induced hypertension (PIH) (8), maternal creatinine, gestational age, intravascular fluid volume, and serum bilirubin (10).

The AKI is defined as a wide spectrum disease classified into three groups for better management and diagnosis: pre-renal, renal, and post-renal. Post-renal AKI can be easily confirmed by renal ultrasonography; nevertheless, it is challenging to differentiate between renal and pre-renal categories based on clinical evidence and laboratory tests, such as urine volume, FENA, Bun to creatinine ratio, and urine sediment (10). Treatment strategies also differ in these two groups. The management of pre-renal AKI is based on fluid replacement, while renal type needs fluid restriction (11-13). The conducted studies illustrated that urine calprotectin is a better marker to distinguish between renal and pre-renal AKI, as compared to neutrophil gelatinase-associated lipocalin (NGAL), Urinary kidney injury molecule-1 (KIM-1), and Fractional Excretion of Sodium (FeNA) (14). Calprotectin is an immune mediator protein that has a protective role in oxidative injuries caused by inflammations. Epithelial cells of collecting ducts also secret calprotectin; therefore, this protein can be found in urine shortly after renal injuries (15). Previous studies have pointed to the relationship of calprotectin in physiologic fluids with feces and inflammatory diseases (16). The number of surveys on neonatal renal failure is limited, in comparison to those performed on adults and even pediatric patients.

Despite the high frequency of AKI among neonates in NICUs, there is a dearth of studies in this regard due to the absence of a unique desirable definition criterion worldwide (17-19). An elevation in creatinine level lags 48-72 h behind renal injury; moreover, it demonstrates the renal function, not renal injury. The measurement of neonatal urine volume is difficult (20, 21); accordingly, neonates usually have nonoliguric renal failure, and oliguria is not a sensitive marker to diagnose AKI in them (22). It is of paramount importance to find another biomarker for the diagnosis of AKI in neonates. In light of the aforementioned issues, the present study aimed to find whether urine calprotectin could be a desirable noninvasive and sensitive biomarker for the diagnosis of AKI in neonates. We also compare calprotectin in pre-renal and renal groups of AKI to assess whether it can be used to distinguish between these two types.

Methods

Design and patients

This cross-sectional study was conducted from March till December of 2018 at Ali Asghar Hospital affiliated with the Iran University of Medical Science. The study population included 100 neonates aged 3-14 days enrolled in the study and assigned to two groups of the case (n=80) and controls (n=20). The neonates in the case group were diagnosed with AKI according to the modified 2016 Kidney Disease Improving Global Outcomes (KDIGO) criteria, while the controls were healthy neonates with no history of renal injury. We included the neonates who received at least 48 h of intravenous fluid before creatinine elevation. All parents gave their written informed consent prior to the study. The exclusion criteria were as follows: neonates older than 14 days and younger than 3 days, gestational age less than 28 weeks, the presence of obstructive uropathies, urinary tract infection, congenital heart disease that needs heart surgery during first 7 days of life, fatal chromosomal anomalies, severe congenital renal anomalies, as well as prenatal risk factors of AKI (including the maternal history of using nephrotoxic drugs, diabetes mellitus, renal disorders, eclampsia, and preeclampsia, chronic hypertension, oligo or polyhydramnios, clinical chorioamnionitis, and vaginal bleeding during pregnancy).

Outcome and Measurements

In the present study, AKI diagnosis was confirmed according to proposed neonatal AKI definition modifications from KDIGO pediatric AKI definition, using serum creatinine (mg/dl) and urine output (ml/kg/h). When serum creatinine rise ≥ 0.3 within 48 h or ≥ 1.5-1.9 rise from baseline (defined as previous lowest value) within 7 days or urine output ≤ 1 for 24 h, it is considered Stage 1. Stage 2 is ≥ 2-2.9 rise of Serum cr from baseline or urine output ≤ 0.5 for 24 h. Stage 3 is defined as increase in serum creatinine by ≥ 0.3 from baseline or ≥ 2.5 or RRT initiation or urine output ≤ 0.3 for 24 h (23). Creatinine at the time of admission is regarded as baseline creatinine. Creatinine is ignored in the first three days of life since it is affected by maternal creatinine.

In order to eliminate the effect of bilirubin on serum creatinine, creatinine in the healthy group was measured by HITACHI E311 auto analyzer. Urine output was measured by diaper weighting or catheter urine collection every 3 h. When AKI was confirmed and before receiving any IV fluid, a 5cc urine sample was collected simultaneously with the blood sample. In the control group, random urine was collected simultaneously with the blood sample upon admission. In both groups, the urine samples were collected by suprapubic aspiration, catheterization, or midstream specimen of urine (MSU) methods. Urine samples were stored at -80C. Calprotectin concentration was measured by Enzyme-linked Immunosorbent Assay (ELISA) according to manufacture protocol. The result was reported in mg/dl. Plasma creatinine was measured and reported in mg/dl.

The following information was collected from hospital records: gender, age, the reason for admission, gestational age, Apgar, intubation, receiving CPR at birth, history of nephrotoxic drugs, plasma creatinine, urine output, type of renal failure, stages of renal failure, maternal history (the used drugs during pregnancy, diabetes mellitus, eclampsia and preeclampsia, chronic hypertension, chronic renal failure, and vaginal bleeding).

Age in this study was defined as passing days after birth, and gender was defined as girl and boy. Weight was considered at birth in kilogram. The specific gravity of urine was measured by a dipstick test. The cause of AKI was categorized into different causes of disease that lead to hospital admissions, such as sepsis, metabolic disorders, respiratory distress syndrome (RDS), hypoxic-ischemic encephalopathy, seizure, needing surgery, prematurity, dehydration, and jaundice. The AKI was classified into three stages base on the modified 2016 KDIGO neonatal AKI definition. To assign the type of AKI, a decrease in serum creatinine after 48 h of fluid replacement therapy was regarded as pre-renal, any obstructive pathologies in renal sonography were considered post-renal, and others were defined as renal. The history of 5 minutes Apgar score, intubation, and CPR in the delivery room was acquired from patient hospital records. Drug information was retrieved from patients' drug sheets. Nephrotoxic drugs were used for all the neonates enrolled in this study, and no interventional management was performed. It was not possible to use FENA to differentiate between renal and pre-renal since diuretics were used for most of the cases during admission. In order to eliminate the evaporation effect of warmers and NICU temperature on urine output, it was measured every 3 h. To reduce the effect of fluid overload that leads to an increase in urine output, neonates were weighed daily. Weight changes are depicted in the table.

Sample size

The sample size was calculated at 80 and 20 for the case and control groups, respectively, based on a study performed by Basiratnia (3) with a sensitivity of 89.7, specificity of 97.1 for calprotectin, the prevalence of 0.4 for AKI in Iranian neonates, a power of 95%, and an alpha error of 0.05.

Ethical considerations

The study was approved by the Iran University of Medical Science Ethics committee (ID number IR.IUMS.FMD.REC1396.9411165024). We explained the study to the parents of participants and obtained parental consent before the commencement of the study.

Statistical Analysis

Mean and standard deviation were used to describe the results of quantitative variables (mean±SD), while the percentage was utilized for qualitative ones. The normality of data was checked using the Kolmogorov-Smirnov (KS) test. Independent T-test and one-way analysis of variance (ANOVA) test were employed to compare the difference between quantitative variables and if there was a statistically significant difference, the Tukey test was used to compare the groups. Qualitative variables were compared using the chi-square test. The receiver operator characteristic curve (ROC) and area under the curve (AUC) were used to determine an appropriate cut-off point of urine calprotectin for AKI diagnosis in neonates. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of urine calprotectin were also calculated. The Kappa agreement coefficient was employed to assess the consistency between the urine calprotectin and gold standard results for AKI diagnosis, determining the severity of the respiratory failure. Data were analyzed in SPSS software (version 20) and STATA software (version 12) (StataCorp, Texas, USA). A p-value less than 0.05 was considered statistically significant.

Results

A total of 100 neonates aged 3-4 days who

were admitted to the NICU of Ali Asghar Hospital participated in this study, including 80 patients with AKI (53 pre-renal and 27 renal) and 20 healthy ones. The gender was not significantly different between the two groups. The majority of the newborns with AKI were at stage one (64%), and the most frequent cause of admission was sepsis (25%). Most of the cases had no history of CPR and intubation (85% and 75% respectively); nonetheless, most of the patients with these histories corresponded to the pre-renal group. Different stages of AKI demonstrated a significant difference in intubation history (P=0.01). There was a significant difference between the stages and types of AKI (P<0.001). In both pre-renal and renal AKI, the majority of cases (64%) corresponded to stage one, followed by stage two (15%) and stage three (1%). The clinical characteristics of the participants are displayed in Table 1. In one-way ANOVA, there was a significant statistical difference for urine specific gravity (SG) among participants (P=0.02). The Tukey test pointed to a meaningful difference between both renal and pre-renal groups (P=0.01) and pre-renal and healthy subjects (P=0.01; Table 2). In the current study, neonates with AKI were significantly older than healthy ones (P< 0.001); however, the Tukey test result showed that this difference was significant between healthy and AKI subjects (P<0.001). Nevertheless, this finding was expected since physiologic icter usually presents on days 2-5 after birth. Serum Creatinine levels in the AKI group were higher than those in neonates without AKI (P< 0.001). Gestational age, birth weight, and Apgar score were not significantly different between the groups (P > 0.05).

Table 1. Demographic characteristics of participants

	Case n=80	Control n=20	ALL n=100	P-value
Gender				
Boy	48 (60 %)	12 (60%)	60 (60%)	0.4
Girl	32 (40%)	8 (40%)	40 (40%)	
Age (mean ± SD)	7.81±3.8	3.4±0.9		<0.001
Gestational age (mean ± SD)	36.2 ± 3.9	37.5 ± 1.5		0.34
Gestational weight (mean ± SD), Kg	2.65±0.9	2.9±0.5		0.35
Apgar (mean ± SD)	8.55 ± 1.2	9.0 ± 0.6		0.36
Stage				
1	64 (80%)	0	64(64%)	
2	15 (18.75%)	0	15(15%)	<0.001
3	1 (1.25%)	0	1(1%)	
Admission cause				
Sepsis	25 (31.25%)	0	25 (25%)	
Hypoxic encephalopathy	7 (8.75%)	0	7 (7%)	
Prematurity	6 (7.5%)	0	6 (6%)	
RDS	14 (17.5%)	0	14 (14%)	<0.001
Seizure	6 (7.5%)	0	6 (6%)	
Metabolic assay	6 (7.5%)	0	6 (6%)	
Dehydration	3 (3.75%)	0	3 (3%)	
Surgery	13 (16.25%)	0	13 (13%)	
Icter	20 (25%)	20 (100%)	20 (20%)	
Intubation				
Yes	25 (31.25%)	0	25 (25%)	0.01
No	55 (68.75%)	20	75 (75%)	
CPR				
Yes	15 (18.75%)	0	15 (15%)	0.09
No	65 (81.25%)	20 (100%)	85 (85%)	

Table 2. Comparing the mean and standard deviation between study and control groups (one way ANOVA test)

	Renal n = 27	Pre renal n = 53	Control n = 20	P value
Serum creatinine (mean ± SD)	0.8 ± 0.2^{b}	0.8 ± 0.4^{b}	$0.4\pm0.08^{\alpha}$	<0.001
Urine SG (mean ± SD)	$1009.7\pm3.7^{\alpha}$	1014.1 ± 7.7^{b}	$1009.3\pm4.0^{\alpha}$	0.02
urine calprotectin (mean ± SD)	153.9±34.2	138.5±25.8	142.4±34.4	0.10
Calprotectin/Urinosmol (mg/mosmol/kg/H2o) ratio	0.45 ± 0.20^{a}	0.34 ± 0.22^{b}	0.76 ± 0.4^{a}	0.007
Fluid therapy duration before sampling(day)	3.52±1.45	3.49±1.22	2.76±1.1	0.59

*Same alphabet demonstrates no statistical relation and different alphabet show a meaningful difference.

Table 3 displays the results of the one-way ANOVA test which compared the effect of different variables on stages of AKI. Serum creatinine levels were significantly different between stages of AKI (P<0.001). A significant statistical difference was demonstrated between urine SG and AKI stages (P< 0.001). The results of the one-way ANOVA test revealed no significant difference in gestational age, birth weight, Apgar score, and even urine calprotectin between the stages of AKI (Table 3).

The ROC curves were used to determine the appropriate concentration of calprotectin for the diagnosis of AKI in newborns. This curve showed the poor discriminatory ability of urine calprotectin to distinguish AKI based on the AUC obtained at 0.512. The optimal calculated cut-off value for urine calprotectin level to predict AKI with a sensitivity of 77.5% and specificity of 40% was 123.5 ng/ml (Figure 1). Table **4** presents a comparison of urine calprotectin with the standard definition of AKI. In 70% of participants, AKI diagnosis concurred with a positive predictive value of 83.78 and a negative predictive value of 30.76.The observed agreement for calprotectin was 0.15 (Kappa=0.15; P=0.11).

Table 3. Comparing the mean and standard deviation between stages of acute kidney injury groups

	Stage3	Stage 2	Stage 1	P value
Gestational age (mean±SD)	40±0	36.0±5.0	36.2±3.5	0.62
Birth weight (mean±SD)	3.20±0	2.5±0.9	2.7±0.8	0.68
Apgar (mean ± SD)	9±0	8.8±1.01	8.5±1.4	0.78
Serum creatine (mean ± SD)	$3.6±0^{b}$	$1.01±0.2^{a}$	0.7±0.1	<0.01
Urine SG (mean ± SD)	$1030±0^{b}$	$1017.6±8.4^{a}$	1011.2±5.6	<0.01
Urine calprotectin (mean ± SD)	104±0	151.6±45.9	142.5±24.3	0.22
Calprotectin/Urinosmol (mg/mosmol/kg/H2o) Ratio	$0.9±0.4^{a}$	$0.29±0.18^{b}$	$0.40±0.22^{c}$	0.03
Fluid therapy duration before sampling(day)	$2.00±1.22^{c}$	$3.40±1.35b$	$3.5±1.48^{a}$	0.58

Table 4. Comparison of urine calprotectin accuracy with a standard definition of acute kidney injury

Gold standard (crt and u/o)					sensitivity	specificity	accuracy	PPV	NPV	Kappa	P-value
		healthy	AKI	total							
Calprotectin	healthy	8	18	26	77.5%	40%	70%	83.7%	30.7%	0.15	0.11
	AKI	12	62	74							
	Total	20	80	100							

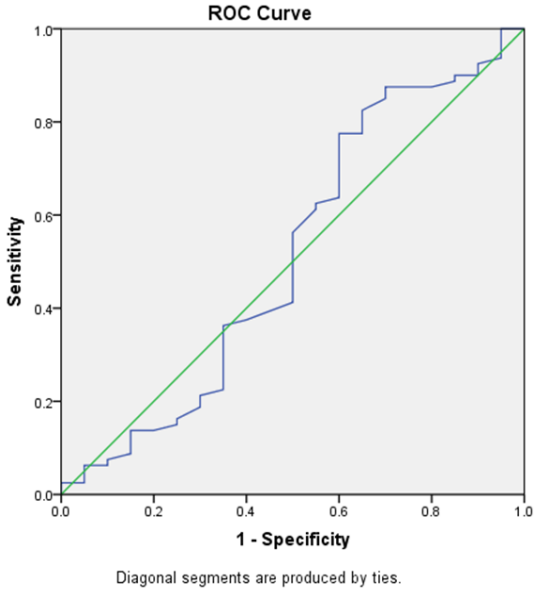

Figure 1. Receiver operating characteristic curve for urine calprotectin level in the diagnosis of acute kidney injury in neonates

Discussion

To the best of our knowledge, it was the first study to compare random urine calprotectin concentration in neonates with AKI and healthy ones. This research aimed to assess the ability of urine calprotectin to diagnose AKI. The obtained results indicate that random urine calprotectin level in neonates is not significantly different between these two groups. It cannot be used as a biomarker for the diagnosis of AKI; moreover, it is

not able to discriminate between renal and pre-renal types. It was found that random urine calprotectin with a cut-off value of 123.5, a specificity of 40%, and a sensitivity of 77.5% can help to distinguish between healthy neonates and those with AKI. In the current study, the level of urine calprotectin was not correlated with the severity of AKI. Limited studies have investigated urine calprotectin as a diagnostic biomarker of AKI in neonates, and the majority of them were carried out on adults and pediatric patients. For instance, in 2016, West Hoff et al. compared urine calprotectin, NGAL, and KIM1 in pediatric patients with confirmed AKI based on KDIGO and PRIFLE. Their study showed that urine calprotectin performance outperformed KIM1 and NGAL in the diagnosis of AKI (24).

In their study, Basiratnia et al. (2017) made a comparison between 75 neonates with AKI and 20 healthy ones. Urine calprotectin was used as a marker to differentiate between acute prerenal and renal failure in neonates. In their study, the mean of urinary calprotectin in renal AKI was 1240 ng/ml versus 285 ng/ml in functional type and 33 in the healthy group, demonstrating a significant difference. The amount of urinary calprotectin was higher in the renal group, while the levels of creatinine and GFR were equals in both renal and prerenal groups ($P<0.05$). In the stated study, urinary calprotectin level was associated with the severity of the renal failure, and its mean was significantly higher in the category of injury and failure of renal failure (3). In the current study, the mean scores of calprotectin were reported as 153.9, 138.5, and 142.5 in the renal, prerenal, and healthy groups. Nonetheless, this difference was not significant, and calprotectin was not recognized as an indicator for the differentiation between renal and prerenal AKI. Therefore, calprotectin with a cut-off value of 230 ng/ml, 95.6% sensitivity, and 100% specificity can be used to differentiate between renal and pre-renal AKI (3).

Seibert et al. in 2016 assessed the power of calprotectin in the differentiation between pre-renal and renal acute allograft. They yielded that urinary calprotectin levels were 36 times higher in subjects with intrinsic AKI than in the pre-renal group (AUC of 0.94) (25). In the same context, Heller et al. (2011) analyzed urine calprotectin to make a distinction between the renal and pre-renal types of AKI. They reported that renal AKI leads to highly increased urine calprotectin concentrations (14). The renal tubular function is immature in neonates, especially in preterm ones. Inappropriate excretion of urinary proteins such as calprotectin could be explained by renal maturity. On the other hand, we used KDIGO criteria to diagnose AKI in this study; nonetheless, the lack of histological confirmation of AKI diagnosis can lead to a discrepancy between the results of the present research and those obtained in other studies conducted on the pediatric and adult population.

In conclusion, there may not be a significant relationship between urine calprotectin level and AKI in neonates; however, it is able to predict AKI in adults and pediatric patients. The achievement of a urine sample in comparison with blood creatinine is much easier and noninvasive. Consequently, anemia is a major cause of neonatal morbidity, especially, preterm neonates admitted to NICU wards. Postponing blood sampling can help to avoid anemia in this group; therefore, it is crucial to find new diagnostic biomarkers with no need for blood sampling.Biomarkers with high sensitivity and specificity for predicting AKI in neonates, such as NGAL have been discovered before. They can improve the detection of AKI and its differential diagnosis, apart from serum creatinine and urine output. It is of utmost importance to find some biomarkers as a reliable measurement tool across different laboratories.

Among the notable limitation of the present study, we can refer to the absence of a diagnostic gold standard definition of AKI in neonates. The latest definition of AKI is based on urine output and serum creatinine; nonetheless, it is not widely accepted in this population since these criteria have some limitations, such as nonoliguric renal failure, difficulties to measure urine output in this group, diuretic therapy in NICUs, the timing of blood sampling, as well as the time lag in creatinine elevation and maternal creatinine which affect neonates creatinine. Furthermore, we could not measure serum creatinine daily; therefore, the exact time of renal failure onset is unclear. Consequently, it is not possible to predict the exact time that calprotectin level starts to rise in the urine. The aforementioned issues can limit our time-related diagnostic ability. Finally, due to inadequate budget, we could not serially measure calprotectin at the time of diagnosis and following that.

Acknowledgments

The authors thank Hoseini, Ph.D., and Gholhak Lab for their assistance performing urine calprotectin testing and the NICU staff of Ali Asghar Children Hospital for helping us in the

collection of urine and blood samples, as well as Ali Asghar Clinical Research Development Center (AACRDC) for editorial/statistical/ and methodological assistance.

Funding

The study was funded by the Iran University of Medical Science.

References

1. Jetton JG, Guillet R, Askenazi DJ, Dill L, Jacobs J, Kent AL, et al. Assessment of worldwide acute kidney injury epidemiology in neonates: design of a retrospective cohort study. *Frontiers in pediatrics*. 2016;4:68.
2. Jetton JG, Boohaker LJ, Sethi SK, Wazir S, Rohatgi S, Soranno DE, et al. Incidence and outcomes of neonatal acute kidney injury (AWAKEN): a multicentre, multinational, observational cohort study. *The lancet child & adolescent health*. 2017;1(3):184-94.
3. Basiratnia M, Kosimov M, Farhadi P, Azimi A, Hooman N. Urinary Calprotectin as a Marker to Distinguish Functional and Structural Acute Kidney Injury in Pediatric Population. *Iranian Journal of Pediatrics*. 2017;27(5).
4. Stojanović V, Barišić N, Radovanović T, Bjelica M, Milanović B, Doronjski A. Acute kidney injury in premature newborns—definition, etiology, and outcome. *Pediatric Nephrology*. 2017;32(10):1963-70.
5. Faa G, Gerosa C, Fanni D, Nemolato S, Locci A, Cabras T, et al. Marked interindividual variability in renal maturation of preterm infants: lessons from autopsy. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2010;23(sup3):129-33.
6. Rakow A, Laestadius Å, Liliemark U, Backheden M, Legnevall L, Kaiser S, et al. Kidney volume, kidney function, and ambulatory blood pressure in children born extremely preterm with and without nephrocalcinosis. *Pediatric Nephrology*. 2019;34(10):1765-76.
7. Hooman N. Is Diagnosis of Acute Kidney Injury Possible in Neonates? *Journal of Pediatric Nephrology*. 2015;3(1):6-.
8. Go H, Momoi N, Kashiwabara N, Haneda K, Chishiki M, Imamura T, et al. Neonatal and maternal serum creatinine levels during the early postnatal period in preterm and term infants. *Plos one*. 2018;13(5):e0196721.
9. Filler G, Guerrero-Kanan R, Alvarez-Elías AC. Assessment of glomerular filtration rate in the neonate: is creatinine the best tool? *Current opinion in pediatrics*. 2016;28(2):173-9.
10. Winterberg PD, Lu CY, Palmer BF. Acute kidney injury: the beginning of the end of the dark ages. *The American journal of the medical sciences*. 2012;344(4):318-25.
11. Al-Ismaili Z, Palijan A, Zappitelli M. Biomarkers of acute kidney injury in children: discovery, evaluation, and clinical application. *Pediatric Nephrology*. 2011;26(1):29-40.
12. Devarajan P. Biomarkers for the early detection of acute kidney injury. *Current opinion in pediatrics*. 2011;23(2):194.
13. Ostermann M, Philips BJ, Forni LG. Clinical review: Biomarkers of acute kidney injury: where are we now? *Critical care*. 2012;16(5):1-13.
14. Heller F, Frischmann S, Grünbaum M, Zidek W, Westhoff TH. Urinary calprotectin and the distinction between prerenal and intrinsic acute kidney injury. *Clinical Journal of the American Society of Nephrology*. 2011;6(10):2347-55.
15. Schrezenmeier E, Barasch J, Budde K, Westhoff T, Schmidt-Ott K. Biomarkers in acute kidney injury–pathophysiological basis and clinical performance. *Acta physiologica*. 2017;219(3):556-74.
16. Rezniczek GA, Förster C, Hilal Z, Westhoff T, Tempfer CB. Calprotectin in pregnancy and pregnancy-associated diseases: a systematic review and prospective cohort study. *Archives of gynecology and obstetrics*. 2019;299(6):1567-77.
17. Ostermann M, Chang R. Challenges of defining acute kidney injury. *QJM: An International Journal of Medicine*. 2011;104(3):237-43.
18. Esfandiar N, Mohkam M, Afjeii A, Kompani F, Shahrazad I, Naderi M, et al. Prognostic factors and mortality rate in neonates with acute renal injury in NICU. *Journal of Pediatric Nephrology*. 2013;1(1):32-6.
19. Tsagalis G. Update of acute kidney injury: intensive care nephrology. *Hippokratia*. 2011;15(Suppl 1):53.
20. Crump C, Sundquist J, Winkleby MA, Sundquist K. Preterm birth and risk of chronic kidney disease from childhood into mid-adulthood: national cohort study. *bmj*. 2019;365.
21. Walker M, Clark R, Spitzer A. Elevation in plasma creatinine and renal failure in premature neonates without major anomalies: terminology, occurrence and factors associated with increased risk. *Journal of Perinatology*. 2011;31(3):199-205.
22. Bruel A, Rozé J-C, Quere M-P, Flamant C, Boivin M, Roussey-Kesler G, et al. Renal outcome in children born preterm with neonatal acute renal failure: IRENEO—a prospective controlled study. *Pediatric Nephrology*. 2016;31(12):2365-73.
23. Zappitelli M, Ambalavanan N, Askenazi DJ, Moxey-Mims MM, Kimmel PL, Star RA, et al. Developing a neonatal acute kidney injury research definition: a report from the NIDDK neonatal AKI workshop. *Pediatric research*. 2017;82(4):569-73.
24. Westhoff JH, Fichtner A, Waldherr S, Pagonas N, Seibert FS, Babel N, et al. Urinary biomarkers for the differentiation of prerenal and intrinsic pediatric acute kidney injury. *Pediatric Nephrology*. 2016;31(12):2353-63.
25. Seibert FS, Rosenberger C, Mathia S, Arndt R, Arns W, Andrea H, et al. Urinary calprotectin differentiates between prerenal and intrinsic acute renal allograft failure. *Transplantation*. 2017;101(2):387-94.