Accuracy of urine calprotectin for diagnosis of acute kidney injury in neonates

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Abstract

Background: Urine calprotectin significantly rises in acute kidney injury (AKI) in adult and pediatrics. The aim of the present study was to investigate the accuracy of urine calprotectin as a diagnostic marker for (AKI) in neonates. **Methods** In this cross sectional study, we assessed urine calprotectin in 100 neonates, in which 80 of them had AKI and 20 were healthy. Random urine calprotectin measured by ELISA and then compared between two groups. We included neonates who had received at least 48 hour intra venous fluid and met our inclusion and exclusion criteria. . Receiver-operating characteristic (ROC) curve used to set a cut of point for urine calprotectin to predict AKI. The overall accuracy and Kappa coefficient was used for assess the agreement between two methods.. P value below 0.05 considered significant. **Results:** Urine calprotectin levels were not significantly higher in neonates with AKI than healthy ones (146.2 vs 142.4, $p=0.1$). The results showed an optimal cutoff value of 123.5 mg/dl for urine calprotectin with area under the curve of 0.515 with sensitivity, specificity, positive predictive value and negative predictive value of 77.5%,40%,83.7% and 30.7%, respectively. The overall accuracy was 70% and the Kappa agreement coefficient was 0.15 ($P=0.11$). **Conclusion:** Although urine calprotectin level can predict the AKI, it is not accurate measure comparing the gold standard.

1. Introduction

Acute renal failure is a common morbidity in neonates who are admitted in neonatal intensive care units (30% worldwide) [1]. Mortality and hospital stay lengths in neonates with AKI is higher than those without AKI [2] and the risk of chronic renal failure in adulthood increased in pediatrics with AKI [3]. It is obvious that prognosis of AKI is highly depended on early diagnosis and onset of treatment. So diagnosis of AKI with respect to physiologic changes in neonates is too important. Nephrogenesis begins from 5th week of gestational age and will be completed till adulthood [4]. There is not enough studies about the effect of prematurity and IUGR on nephrogenesis, but adverse effect of renal failure on renal maturation is obvious [5, 6] Definition of AKI for neonates, in neonatal AKI workshop which was held in 2017, was modified from pediatrics definition based on serum creatinine and urine output[7]. Serum creatinine in neonates could be affected by maternal creatinine, gestational age, intra vascular fluid volume and serum bilirubin. Aki define as wide spectrum disease that for better management and diagnosis classified in three groups: prerenal, renal and post renal. Post renal AKI can easily confirmed by renal ultrasonography but differentiation between renal and pre renal category based on clinical evidence and laboratory tests such as urine volume, FENA, Bun to creatinine ratio and urine sediment is challenging [8]. Treatment strategies is also different in these two. Management of pre renal AKI is based on fluid replacement, while renal type needs fluid restriction [9-11]. Studies showed urine calprotectin is a better marker than NGAL and KIM1 and FeNA to distinguish renal from pre renal AKI [12]. Calprotectin is an immune mediator protein that has a protective role in oxidative injuries due to inflammations. Epithelial cells of collecting ducts also secrets calprotectin. So this protein can find in urine soon after renal injuries [13]. In former studies the relation between calprotectin in physiologic fluids and feces and inflammatory disease has shown [14]. Number of surveys on neonatal's renal failure is more fewer than in adults and even pediatrics. Despite

high frequency of AKI among neonates in neonatal intensive care units, there isn't enough studies about it because the lack of a unique desirable definition criteria worldwide [15-17]. Serum creatinine rise lagged about 48-72 hours after renal injury and also it shows the renal function, not renal injury. measuring neonatal urine volume is difficult [18, 19]. Hence, neonates usually have non oliguric renal failure and oliguria is not a sensitive marker to diagnose AKI in them [20]. So finding another biomarkers for diagnosis of AKI in neonates is crucial. The aim of this study is to find whether urine calprotectin could be a desirable noninvasive and sensitive biomarker for diagnosis of AKI in neonates. We also compare calprotectin in prerenal and renal groups of AKI to assess whether it can use to distinguish between these two types.

2. Materials And Methods

2.1. Design and patients

This is a cross sectional study that conducted during March till December of 2018 at Ali Asghar children hospital affiliated to Iran University of medical science. Study group consisted of 100 neonates aged 3 to 14 days enrolled the study, which diagnosis of AKI according to modified 2016 KDIGO neonatal AKI definition were confirmed for 80 of them and 20 healthy neonates with no history of renal injury that admitted for receiving phototherapy because of physiologic icter as a control group. We included neonates who received at least 48 hour intra venous fluid. Information about sex, age, cause of 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 (drugs using during pregnancy and drug consumption, diabetes mellitus, eclampsia and preeclampsia, chronic hypertension, chronic renal failure, vaginal bleeding were collected by questionnaire from hospital records. Neonates older than 14 days and younger than 3 days, suffering of obstructive uropathies, UTI, congenital heart disease that need heart surgery during first 7 days of life, fatal chromosomal anomalies, severe congenital renal anomalies, having prenatal risk factors of AKI (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) were excluded from the study.

2.2. Data sources and measurement

AKI diagnosis was 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 day or urine output ≤ 1 for 24 h consider as Stage 1. Stage 2 is ≥ 2 -2.9 rise of Serum cr from baseline or urine output ≤ 0.5 for 24 h. Stage 3 defined as serum creatinine ≥ 3 rise from baseline or ≥ 2.5 or RRT initiation or urine output ≤ 0.3 for 24 h. Creatinine at the time of admission consider as base line creatinine. First 3 day of life creatinine is ignored because it is affected by maternal creatinine. In order to eliminate the effect of

bilirubin on serum creatinine, creatinine in healthy group measured by HITACHI E311 auto analyzer. Urine output measured by diaper weighting or catheter urine collection every 3 hours. After receiving IV fluid for at least 48 hours, from patients that AKI was confirmed for them, 5cc urine sample collected simultaneously with blood sample at the time that AKI were confirmed. In control group random urine collected at the time of admission simultaneous with blood sample. Urine samples were stored at -80C. Calprotectin concentration measured with immunosorbent enzyme linked ELISA kit according to manufacture protocol. The result reported in mg/dl. Plasma creatinine measured and reported in mg/dl. Age in this study defined as passing days after birth. Sex defined as girl and boy. Weight at birth time in kilo gram. Specific gravity of urine measured by dipstick test. Cause of AKI categorized in different causes of disease that lead to hospital admission such as sepsis, metabolic disorders, RDS, hypoxic ischemic encephalopathy, seizure, needing surgery, prematurity, dehydration and icter. AKI classified in 3 stages base on modified 2016 KDIGO neonatal AKI definition. To assign type of AKI, decrease in serum creatinine after 48 hours of fluid replacement therapy considered as pre renal, any obstructive pathologies in renal sonography as post renal and others consider as renal. History of 5 minute Apgar score, intubation and CPR in delivery room was acquired from patient hospital records. Drugs information acquired from patients drug sheets. Nephrotoxic drugs were used for all the neonates that enrolled in this study. No interventional management was held in this study. We cannot use FENA to differentiate renal from pre renal because diuretics were used for most of the cases during admission. In order to eliminate evaporation effect of warmers and NICU temperature on urine output, we measured it every 3 hour. To reduce the effect of fluid over load that lead to increase in urine output, neonates weighted daily.

2.3. Sample size

sample size was calculated based on BASIRATNIA α 's study [7] with sensitivity of 89.7 and specificity of 97.1 for calprotectin and prevalence of 0.4 for AKI in Iranian neonates, and using a power of 95% and an alpha error of 0.05, sample size for case group designed 80 and for control group was 20.

2.4. Ethical considerations

The study was approved by Iran University of medical science Ethics committee (ID number IR.IUMS.FMD.REC1396.9411165024). We explained the study to the parents of participant and obtained parental consent before starting the study.

2.5. Statistical Analysis

Mean and standard deviation used to describe results of quantitative variables (mean SD) and percentage used for qualitative ones. Independent T-test and one way analysis of variance (ANOVA) test used to compare the difference between quantitative variables and if there was statistically significant, Tukey test used to compare the groups. Qualitative variables compared with chi square test.. Receiver operator characteristic curve (ROC) and area under the curve (AUC) used to determine an appropriate cut of 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 used to evaluate the consistency between the urine calprotectin and gold standard results for AKI diagnosis determining the severity of respiratory failure. Data were analyzed using SPSS v.20 and STATA software, version 12 (StataCorp, Texas, USA). The $P < 0.05$ was considered as the level of significance in all analysis.

3. Results

Total number of 100 neonates aged three to 14 days who were admitted in Ali Asghars neonatal intensive care unit participated in the study, including 80 patient with AKI (53 prerenal and 27 renal) and 20 healthy. Clinical characteristics of participants were shown in Table 1. The table demonstrates that number of boys is more than girls in this study (60% boy) , but gender were not significantly different between two groups.($p = 0.4$). Most of the newborns with AKI were at stage one (64%) and the most frequent cause of admission was sepsis (25%) and the less was dehydration (3%). Most of the cases have no history of CPR and intubation (85% and 75% respectively), although most of the patient with these histories corresponds to prerenal group. Different stages of AKI showed a significant difference in having intubation history ($p=0.01$). There was a significant difference between stages of AKI and types of it ($p<0.001$). In both prerenal and renal AKI majority of cases corresponded to stage one (64%), followed by stage two (15%) and stage three (1%).

In one way ANOVA analysis, there was significant statistical difference for urine SG among participants ($p= 0.02$). Tukey test showed not only there was a meaningful difference between renal and pre renal group ($p=0.01$), also there was between pre renal and healthy subjects ($p=0.01$) (Table2). Although, no difference between renal and healthy ones was confirmed regarding to urine SG ($p=0.9$). In our study neonates with AKI were significantly older than healthy ones ($p<0.001$), but Tukey test resulted that it is significant between healthy and AKI subjects ($p <0.001$), not between renal and pre renal ones ($p=0.9$). Nevertheless, this finding was expected because physiologic icter usually presents in day 2 to 5 after birth. Serum Creatinine levels in the AKI group were higher than in neonates without AKI ($p<0.001$). But, there is not significant between AKI subtypes ($p=1.0$). Median urine calprotectin levels were 146.2 in the AKI group versus 142.4 in the non-AKI group, but the results were not significantly different ($p=0.1$). Gestational age, birth weight and Apgar score were not significantly different between the groups ($p > 0.05$, for each).

Table3 shows one way ANOVA test results that compare the effect of different variables on stages of AKI. Serum creatinine levels were significantly different between stages of AKI ($p<0.001$). Also results indicated the difference between stage one and stage two ($p<0.001$). Significant statistical difference were demonstrated between urine SG and stages in AKI ($p<0.001$). Moreover, there was significant difference between stage one and stage two ($p=0.01$). However, the results of one way ANOVA test revealed no significant difference in gestational age, birth weight, Apgar score and even urine calprotectin between stages of AKI (Table 3).

Receiver operating characteristic (ROC) curves were used to determine appropriate concentration of calprotectin for diagnosis of AKI in newborns. (figure1) ROC curve showed poor discriminatory ability for urine calprotectin with obtained area under the curve of 0.512 to distinguish AKI. The optimal calculated cut off value for urine calprotectin level to predict AKI with sensitivity of 77.5% and specificity of 40% was 123.5 ng/ml. Table 4 shows Comparison of urine calprotectin with standard definition of AKI. In 70% of participant, AKI diagnosis was concurred with positive predictive value of 83.78 and negative predictive value of 30.76. The observed agreement for calprotectin was 0.15 (Kappa=0.15, p=0.11).

4. Discussion

This is the first study to compare random urine calprotectin concentration in neonates with AKI and healthy ones. The purpose of this study was to assess the ability of urine calprotectin for diagnosis of AKI. Our results indicate that random urine calprotectin level in neonates is not significantly different between these two groups, and not solely can it use as a biomarker for diagnosis of AKI, also can it discriminate renal from prerenal type. We found that random urine calprotectin with cut off value of 123.5 with specificity of 40% and sensitivity of 77.5% can help to discriminate healthy neonates from those with AKI. In our study the level of urine calprotectin were not correlated with severity of AKI.

Our study was limited by 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, but it is not widely accepted in this population, because these criteria have limitations such as non oliguric renal failure which happened in neonates and difficulties to measure urine output in this group and diuretic therapy in NICU, timing of blood sampling and lagged in creatinine rise and maternal creatinine which have effect on neonates creatinin . We couldn't measure serum creatinine daily, so the exact time of renal failure onset is unclear. Thus we are unable to predict the exact time that calprotectin level start to rise in urine. These can limits our time-related diagnostic ability. Because of inadequate budget we could not measure calprotectin serially at the time of diagnosis and then after. Limited studies have investigated urine calprotectin as a diagnostic biomarker of AKI in neonates. The majority were done in adult and pediatrics. In 2016, West hoff et al compared urine calprotectin, NGAL and KIM1 in pediatrics whom diagnosis of AKI was confirmed based on KDIGO and PRIFLE. Their study showed urine calprotectin performance was much better than KIM1 and NGAL for diagnosis of AKI [21]. Basiratnia et al. conducted the study in 2017 to investigate the accuracy of urine calprotectin in discriminating renal from prerenal AKI in pediatrics. They found that urine calprotectin in renal group was higher than pre renal and calprotectin level correlated with severity of renal failure. Hence, Calprotectin with cut off value of 230 ng/ml with 95.6% sensitivity and 100% specificity can use to differentiate renal from prerenal AKI[3]. Seibert et al. in 2016 assessed the power of calprotectin in differentiation of pre-renal and renal acute allograft. They yield that urinary calprotectin level were 36 times higher in subjects with intrinsic AKI than in pre-renal group (AUC of 0.94) [22]. Heller et al. in 2011, analyzed urine calprotectin to distinct renal from pre renal type of AKI and find out renal AKI leads to highly increased urine calprotectin concentrations[12].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 use KDIGO criteria to diagnose

AKI in this study but Lack of histological confirmation of AKI diagnosis in our study, can lead to discrepancy when our results compare to other studies that conducted in pediatric and adult population.

In conclusion there is not significant relation between urine calprotectin level and AKI in neonates. However it is enable to predict AKI in adults and pediatrics. Achieving urine sample in comparison with blood creatinine is much easier and also noninvasive. Hence anemia is one of the important comorbidity for neonates, specially, pre terms who were admitted in NICU wards that can affect their growth. Hesitating blood sampling can help to avoid anemia in this group. So finding new diagnostic biomarkers with no need of blood sampling could be very crucial in this group. Biomarkers with high sensitivity and specificity for predicting AKI in neonates like urine neutrophil Gelatinase -associated lipocalin (NGAL) were discovered before. They can help beside serum creatinine and urine output to improve detecting of AKI and its differential diagnosis. It will be important to find biomarkers to be a reliable measurement tools across different laboratories.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Conception or design of the work: NK, SM, NH and MK.

Data collection: SM, NK.

Data analysis and interpretation: MK

Drafting the article: NK, SM, NH, MK.

Critical revision of the article: NK, SM, NH and MK.

Final approval of the version to be published: All authors read and approved the final manuscript.

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Figures

Figure 1- Receiver operating characteristic curve for urine calprotectin level in diagnosis of AKI in neonates

Figure 1

Receiver operating characteristic curve for urine calprotectin level in diagnosis of AKI in neonates

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