The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89, Page 5586- 5590

Assessment of Urinary Calprotectin in Early Diagnosis of Intrinsic Acute Kidney Injury in Critically Ill Children at Zagazig University Hospitals

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ABSTRACT
Background: Acute kidney injury (AKI) is a common and potentially life-threatening condition. AKI is defined by an increase of serum creatinine by ≥0.3 mg/dL in 48 h or an increase by ≥1.5-fold from a known or assumed baseline or by a decrease of urinary output to less than 0.5 mL/kg/1 hour (h) for 6 h.
Objectives: To assess the specificity and sensitivity of urinary calprotectin in early detection of intrinsic AKI.
Patients and Methods: This was a cross sectional study that was conducted on 100 children in Pediatric Intensive Care Unit in the Department of Pediatrics, Zagazig University Hospitals.
Results: In this study, 39% of our cases were diagnosed with AKI. The current study showed that, regarding urinary calprotectin on first and third day of admission, there was statistically significant increase in calprotectin level when comparing first and third day levels; p value less than 0.001. Regarding validity of Urinary (U) calprotectin, the value of sensitivity was (76.9%), specificity= (76.9%), PPV = (81.1%), NPV = (81.1%), and (79.5%) accuracy.
Conclusion: The results of our study show that urinary calprotectin has higher sensitivity and specificity than serum creatinine levels for detecting early stages of intrinsic AKI.
Keywords: Urinary Calprotectin, Biomarker, Early Diagnosis, Intrinsic Acute Kidney Injury.

INTRODUCTION

Acute kidney injury (AKI) is a common and potentially life-threatening condition. AKI is defined by an increase of serum creatinine by ≥0.3 mg /dL in 48 h or an increase by ≥1.5-fold from a known or assumed baseline or by a decrease of urinary output to less than 0.5 mL/kg/1 h for 6 h **(1).** It is associated with elevated short-term morbidity and mortality as well as with unfavourable long-term outcomes caused by the development of chronic kidney disease (CKD) or the occurrence of cardiovascular events **(2).**

At the time of AKI diagnosis, a number of diagnostic and therapeutic measures are needed. These measures include the determination of the underlying cause of AKI and the initiation of specific and supportive therapeutic measures, such as antibiotic therapy for sepsis, immunosuppression for autoimmune disease, an adjustment of nephrotoxic drugs or directed fluid management **(3).**

The patients with the most elevated creatinine could be those with the best muscle mass, that is the best health status at baseline. Long periods of azotemia may correlate with time-consuming repair of the renal tubule, while short periods of azotemia may correlate with rapidly reversible hemodynamic variation. In addition, the lack of specificity is the concern over sensitivity because a healthy renal reserve would blunt the rise in creatinine. In fact, removal or damage of a portion of a kidney may not elevate serum creatinine, despite loss of renal mass **(1)**.

One major advance to detect AKI at an earlier stage would be the implementation of new reliable biomarkers that identify AKI earlier than conventional tests or that detect subclinical AKI **(2)**.

Calprotectin has been identified as an antimicrobial protein in the cytoplasm of neutrophil granulocytes. Intracellular calprotectin's main function is to interact with the cytoskeleton whereas when is secreted by activated immune cells it acts as a danger-associated molecular pattern protein **(4)**.

There are many studies investigating the diagnostic accuracy of calprotectin in its ability of distinguishing pre-renal from intrinsic AKI. Calprotectin showed a very high accuracy in predicting intrinsic AKI with an AUC ranging from 0.92 to 0.97 in these studies **(5)**.

The objectives of this study were to assess the specificity and sensitivity of urinary calprotectin in early detection of intrinsic AKI.

PATIENTS AND METHODS

I- Technical design:

Site of the study: Pediatric Intensive Care Unit in the Department of Pediatrics, Zagazig University Hospitals.

Sample size: Comprehensive sample as number of cases admitted with inclusion criteria did not exceed 10 cases per month so in study period of 10 months, we included all of them, which equaled 100.

Type of the study: cross sectional study.
Tools and instruments: Records of the patient, urine output, blood pressure, serum creatinine, Routine lab and specific investigation, which was for urinary calprotectin.

Inclusion criteria: All children from 6 month till 15 years old admitted to the Pediatric Intensive Care Unit (PICU) in Zagazig University Hospital.

Exclusion criteria: Patients with urinary tract obstruction. Patients with preexisting primary renal disease. Patients with systemic diseases with renal

Received: 20/6/2022
Accepted: 28/8/2022

involvement. Patients known with systemic hypertension diagnosed before admission. Patients with inflammatory bowel disease (IBD).

II- Operational design:

Steps of performance and techniques used:

(1) History taking.

(2)General examination (Anthropometric measurements and vital signs).

(3) Systemic examination (Cardiac, chest, abdomen, musculoskeletal examination).

(4) Urine output collection.

(5) Laboratory investigations: in addition to routine lab (CBC, CRP, liver function tests, kidney function tests, PT, PTT, INR and urine analysis) and the specific investigation that was measured was the urinary calprotectin.

N.B. all cases urine sample were taken on admission and full labs and urine sample were also taken at day 3, if creatinine of cases was high than base line, according to KIDGO criteria.

We found 39 cases had AKI, then we take 39 controls group.

N.B., we took control group to know cut off value of calprotectin because it was not made in previous studies.

Table (1): Kidney disease: Improving Global Outcomes (KDIGO) staging of acute kidney injury (AKI3) [6].

Stage	Serum creatinine	Urine output
1	Increase by 1.5–1.9 times baseline within 7 days OR Increase by ≥0.3 mg/dL (26.5 µmol/L) within 48 hours	Less than 0.5 mL/kg/hour for 6–12 hours
2	Increase by 2–2.9 times baseline	Less than 0.5 mL/kg/hour for ≥12 hours
3	Increase by ≥3 times baseline OR Increase to ≥4 mg/dL (353.6 µmol/L) OR Renal replacement therapy initiation OR In patients younger than 18 years, decrease in estimated glomerular filtration rate (GFR) to <35 mL/min/1.73 m^2	Less than 0.3 mL/kg/hour for ≥24 hours OR Anuria for ≥12 hours

Human (CALP) calprotectin ELISA Kit (USA): Principle of the Assay this kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto well plates and the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of target can be calculated.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every caregiver of each patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the mean ± SD and median (interquartile range), and qualitative data were expressed as absolute frequencies (number) and relative frequencies (percentage). Independent samples Student's t-test was used to compare between two groups of normally distributed variables while Mann Whitney U test was used for non-normally distributed variables. Percent of categorical variables were compared using Chi-square test. Validity was calculated for both calprotectin and creatinine to detect their ability to predict AKI. P-value < 0.05 was considered statistically significant.

RESULTS

Regarding demographic characteristics, the median age (years) of the cases group was 1.5 (0.7-4.88), median weight was 11 (6-16.75), the mean height was 89.93±23.87 and the mean Glascow Coma Scale (GCS) was 12.58±2.34. Regarding blood pressure the mean diastolic BL/P was 44.79±13.38 mmHg and the mean systolic BL/P was 76.8±15.43 mmHg. The mean Temperature was 37.7±3.68, the mean heart rate was 138.53±25.36, the median respiratory rate was 32 (23.25-40) and the mean O_2 saturation was 87.51±8.74. About (52%) of case were males and the other (48%) were females. (46%) of cases presented with edema, (45%) presented with cyanosis and only (3%) presented with pallor, more than half of patients needed to use MV, (45%) used nephrotoxic agent, as shown in table (2)

Table (2): Patients basic demographic data of the studied group at time of admission and other data

Variables		Study group (n=100)	
Age (years) Mean ±SD Median (IQR)		3.09±3.26 1.5 (0.7-4.88)	
Sex	Male	**No.**	**(%)**
		52	52
	Female	48	48
Weight (kg) Mean ±SD Median (IQR)		13.28±3.21 11 (6-16.75)	
Height (cm) Mean ±SD Range		89.93±19.61 (60-155)	
GCS Mean ±SD Range		12.58±2.34 (6-15)	
Blood pressure (mmHg) Mean ±SD Range	Systolic BP	76.8±15.43 (50-120)	
	Diastolic BP	44.79±13.38 (20-80)	
Heart rate (beat/min) Mean ±SD Range		138.53±25.36 (85-210)	
RR (cycle/min) Mean ±SD Median (IQR)		32.92±7.91 32 (23.25-40)	
Temperature© Mean ±SD Range		37.7±3.68 (37-38)	
O_2 saturation Mean ±SD Range		87.51±8.74 (65-99)	
Clinical presentation	Pallor	3	3
	Cyanosis	45	45
	Edema	46	46
Use of MV	No	48	48
	Yes	52	52
Use of nephrotoxic agent	No	55	55
	Yes	45	45

About (39%) of cases diagnosed with AKI as shown in table (3).

Table (3): Incidence of development of AKI within the studied groups within 48 h of PICU admission

Variables		Study group (n=100)	
		No.	**(%)**
	With AKI	**39**	**39**
	Without AKI	**61**	**61**

This table shows that there were no statistically significant differences between the studied groups as regard age and sex as shown in table (4).

Table (4): Relation between development of AKI and demographic data of the studied groups

Variables		Group without AKI (n=61)		Group with AKI (n=39)		P value
		No.	**(%)**	**No.**	**(%)**	
Sex	Male	36	59	16	41	
	Female	25	41	23	59	0.079
Age (Years) Mean ±SD Median (IQR)		2.69±2.86 1.45 (0.53-4)		3.73±3.75 2 (1-5.5)		0.066

There was statistically significant increase in urinary calprotectin level when comparing first and third day levels as shown in table (5).

Table (5): Urinary calprotectin versus serum creatinine of the AKI group on first and third day of admission

Variables	Serum. creatinine (mg/dL) Mean ±SD
On first day of admission	0.46±0.11
On third day of admission	1.26±0.29
P value	**<0.001**

Regarding validity of urinary calprotectin, the value of sensitivity was (76.9%) and of specificity was (76.9) as shown in table (6) and figure (1).

Table (6): Validity of urinary calprotectin as a marker in early detection of intrinsic acute kidney injury

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Urinary calprotectin	0.709	0.585-0.834	56.5	76.9%	82.1%	81.1%	78%	79.5%

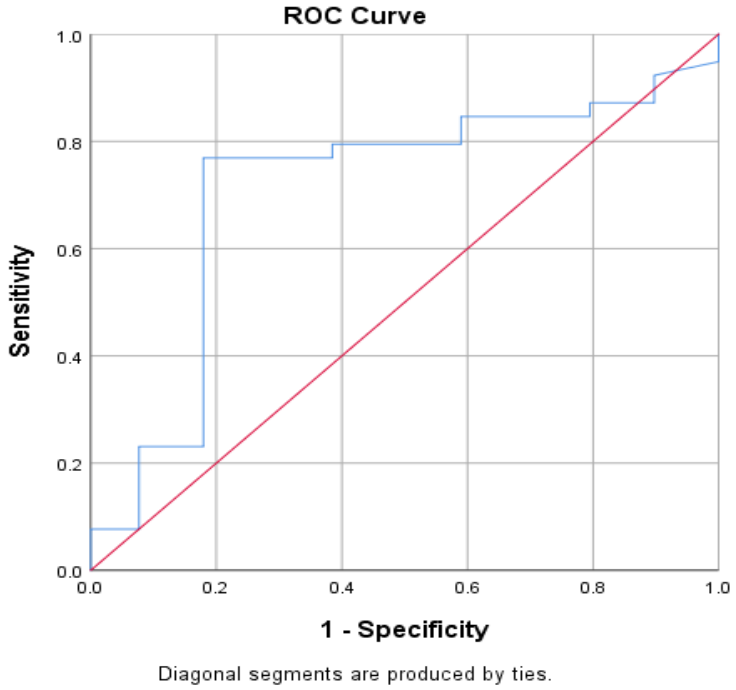

Figure (1): ROC curve for Validity of U. calprotectin.

DISCUSSION

Acute kidney injury (AKI) is a clinical syndrome characterized by the inability of the kidneys to excrete nitrogenous and other waste products, maintenance of fluid and electrolytes balance, and acid-base hemostasis [(3)].

In gastrointestinal problems, fecal calprotectin level differentiate between inflammatory bowel disease from functional intestinal problems as this marker is raised only in inflammatory intestinal problems[(5)]. Therefore, in this study we planned to use urinary calprotectin to detect its sensitivity and specificity in AKI.

52% of our cases were males and the others (48%) were females. This is in accordance with **Mortazavi *et al.*** [(7)] from Tabriz, Iran, who reported male predominance.

In this study, 39% of our cases were diagnosed with AKI. This agreed with **Vakili *et al.*** [(4)] who aimed to compare the sensitivity and specificity of urinary calprotectin with those of serum creatinine in detecting early intrinsic AKI. Over 6 months period (April to October 2018), 81 of 408 patients admitted to the Pediatric Intensive Care Unit met the criteria of this cross-sectional study. Of the total 81 patients, 67 had the criteria of intrinsic AKI.

This agreed with **Abdulsamea *et al.*** [(8)] who aimed to evaluate the incidence of acute kidney injury in intensive care unit patients, to categorize the severity of the acute kidney injury according to the Pediatric Risk (R), Injury (I), Failure (F), Loss, End-Stage (pRIFLE), and to analyze outcome predictors. They reported that the incidence of AKI in their study was (33%). While **Basu, *et al.*** [(9)] reported that incidence rate of AKI in children admitted to pediatric intensive care units (PICUs) ranged from 8% and 89%.

Our study showed that, there were no statistically significant differences between the studied groups as regard age and sex. This agreed with **Paramastuty *et al.*** [(10)] who found that, there was no statistical significant difference between the studied groups regarding sex.

The present study showed that, there were statistically significant differences between the studied groups as regard urinary calprotectin on the first day of admission where the higher mean values were in cases with AKI. This was in agreement with **Vakili *et al.*** [(4)].

The present study showed that, regarding validity of urinary calprotectin, the value of sensitivity was (76.9%), specificity= (76.9%), PPV = (81.1%), NPV= (81.1%), and (79.5%) accuracy.

Gao *et al.* [(11)] have shown that the level of calprotectin was correlated with the degree of sepsis severity, with

an AUROC of 0.901 and a sensitivity and specificity of 83.1% and 88.5%, respectively. They demonstrated that calprotectin levels were significantly higher in patients with septic AKI and in non-survivors at 28 days than in those not meeting these conditions. **Vakili *et al.* [4]** concluded that the sensitivity and specificity of calprotectin levels to diagnose intrinsic AKI in children is 92.5% and 92.8%, respectively. Also, it's positive and negative prognostic value is 98.4% and 72.2%, respectively. The cut point of 530 had the highest point of sensitivity and specificity in their study.

Our results are in accordance with smaller studies performed in the past. The previous studies focused on the value of calprotectin in the differentiation of prerenal from intrinsic AKI [12]. Their study showed that urinary calprotectin has a good performance by a cutoff value of 230 ng/mL, in differentiation of structural and functional AKI in pediatric population with high sensitivity (95.6%) and specificity (100%).

CONCLUSION

The results of our study show that urinary calprotectin has higher sensitivity and specificity than serum creatinine levels for detecting early stages of intrinsic AKI.

Conflict of interest: The authors declare no conflict of interest.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

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