Urinary Calprotectin as A Biomarker of Early Diagnosis of Intrinsic Acute Kidney Injury in Critically Ill Children

[a]HanyElsayed Ibrahim, [b]Naglaa Ali Khalifa[c]Rehab Afifi Gouda Afifi
[d]Ahmed Hosni Mowafy

[a]Professor of pediatrics, Faculty of Medicine – Zagazig University,[b] Professor of Clinical Pathology, Faculty of Medicine – Zagazig University, [c] MB, Bch, Zagazig University[d]Lecturer of Pediatrics, Faculty of Medicine – Zagazig University.

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/1 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 hfor 6 h.The objectives of this study were to assess the specificity and sensitivity of urinary calprotactin in early detection of intrinsic AKI and to compare between Urinary Calprotectin and serum creatinine for early detection of intrinsic AKI**. 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:**About (52%) of our cases were males and the other (48%) were females. In this study, 39% of our cases diagnosed with AKI. Our study showed that, regarding kidney function on first day of admission, the median of each UOP was 2 (1.5-3) (ml/kg/hr), Creatinine was 0.4 (0.23-0.6) and BUN was 16.5 (10-25). Kidney function on third day of admission, the median of each UOP was 3 (1.5-3) (ml/kg/hr), Creatinine was 0.5 (0.3-1.08) and BUN was 20 (12-28). The current study revealed that, only (5%) needed dialysis. There was statistically significant differences between the studied groups as regard Need of dialysis as (12.8%) of cases with AKI needed dialysis. Our study showed that, there were no statistically significant differences between the studied groups as regard age and sex. In this study, there were statistically significant differences between the studied groups as regard Creatinine on the third day of admission where the higher mean values were cases with AKI. The current study showed that, regarding urinary calprotectin on first day of admission, the median was 214 (60-505). Regarding urinary calprotectin on third day of admission, the median was 416.5 (88-1267). There was statistically significant increase in calprotein level when comparing first and third Day levels p value less than 0.001. The present study showed that, regarding validity of U. calprotectin, the value of Sensitivity was (76.9%), specificity= (76.9%), PPV = (81.1%), NPV = (81.1%), and (79.5%) accuracy. **Conclusion:**Urinary calprotectin has higher sensitivity and specificity than serum creatinine levels for detecting early stages of intrinsic AKI. It's early rising in urine allows us to commence our treatments at earlier stages preventing serious kidney tissue damages in children.

Key words: Urinary Calprotectin-Biomarker –Intrinsic Acute Kidney Injury - Critically Ill Children
DOI Number: 10.14704/nq.2022.20.12.NQ77055 **NeuroQuantology 2022; 20(12): 712-723**

Corresponding Author Name: **Rehab Afifi Gouda Afifi**
Phone Number: 01096623254 Email: rehabafify92@gmail.com

Introduction: 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. The incidence of AKI appears to increase recently and its etiology is changing from primary parenchymal disease to multifactorial causes.

Diagnosis of early phases of AKI and initiation of appropriate therapeutic interventions are of utmost importance to prevent the progression of this critical clinical state and improving its prognosis. Efforts had been made to diagnose and classify different phases of AKI which have largely been dependent upon serum creatinine and urine volume changes.

eISSN 1303-515

Prolonged prerenal and postrenal types of AKI also, can lead to parenchymal and renal tissue damage [(1).] According to KDIGO (Kidney Disease Improving Global Outcome) clinical practice guideline for AKI, it is any situation that can lead to an increase in serum creatinine or decrease of urine output or any combination of them. Nowadays serum creatinine and glomerular filtration rate (GFR) are the main laboratory parameters used to diagnose intrinsic AKI. Imaging studies such as ultrasound examinations greatly help us to detect post-renal causes, but differentiation of pre-renal and intrinsic types may be very difficult. Clinical manifestations of early phases of any type of AKI may be mild and trivial. Decreased urine output has a low sensitivity and specificity in the early stages and GFR has to be decreased at least 30% before the onset of any serum creatinine rise. Besides, there is a relatively long latency between any change in GFR level and increase of serum creatinine e.g., after an abrupt decrease in GFR, it will take at least 1–3 days to serum creatinine raising. Also, the results of any treatment to lower serum creatinine will be lately detectable after GFR returns to normal [(2).]

Besides, serum urea is not a constant finding and has considerable false positive (e.g., patients on protein-rich diets, any tissue injury, hemorrhages, trauma, and following glucocorticoids therapy) and false negative (e.g. low-protein diet and advanced liver disease) results which hinder its use in detection of AKI. Any biomarker of AKI should have such accuracy and high specificity and sensitivity to help us detect the disease process at its earlier stage, leading to in time appropriate therapeutic interventions preventing further renal tissue damage. There are more than 20 AKI biomarkers already studied. An ideal biomarker should be one that could be easily measured, with no interference with other biologic variables, and be able to detect early phases of kidney damage. The most common biomarkers studied are neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), cystatin-C, L type fatty acid-binding protein (L-FABP), N-acetyl-beta-D glucosaminidase (NAG), netrin-1, vanin-1, and monocyte chemoattractant protein-1 (MCP-1). NGAL and L-FABP are detectable in earlier and KIM-1 and IL-18 in later stages, with higher specificity [(3).]

In the recent era, there should be a greater impact on clinical practice to detect renal problems much earlier to start treatments when they are more effective. Each of these biomarkers are very valuable, especially in ischemic renal injuries, both experimentally and clinically. Combining different biomarkers is more promising, especially because of the availability of improved methods to validate and quantitate them. The importance of calprotectin among these biomarkers is in its unique properties that make it more practical everywhere [(4).]

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

Patients and Methods

I- <u>Technical design:</u>

Site of design: 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 talked all of them which equal 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 is urinary calprotectin.

Inclusion criteria: All children from 6 month till 15 years old admitted in 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 involvement.Patients known with systemic hypertension diagnosed before admission.Patients with inflammatory bowel disease (IBD).

II- <u>Operational design:</u>

Steps of performance and techniques used:

1. History taking
2. general examination (Anthropometric Measurements & 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 Test, Kidney Function Test, PT. PTT INR and Urine Analysis) and the specific

eISSN 1303-515

investigation that was measured was the urinary calprotectin.

N.B. all cases urine sample were taken on admission and full labs and urine sample is 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 take control group to know cut off value of calprotectin because it was nt made in previous studies.

N.B. we follow this policy to reduce cost.

HumancalprotectinELISAKit

Principle of the Assay:

Thiskitwasbasedon sandwichenzyme-linkedimmune-sorbentassay technology.Captureantibody waspre-coatedonto96-wellplates.And thebiotinconjugatedantibody wasusedas detectionantibodies.Thestandards, testsamplesand biotinconjugateddetectionantibodywereadded tothewellssubsequently, and washedwithwashbuffer.HRP-Streptavidinwasaddedandunboundconjugates werewashedaway with washbuffer. TMBsubstrateswereusedtovisualize HRPenzymaticreaction.TMBwascatalyzedby HRPtoproduceabluecolorproductthatchangedi ntoyellowafteraddingacidicstopsolution.Thede nsity ofyellow is proportionalto thetargetamountofsamplecapturedin plate. Read theO.D.absorbanceat450nm inamicroplatereader,andthentheconcentrationo ftargetcanbecalculated.

Ethical considerations:

1. Risk benefit assessment, all patients will not be subjected to risk of any kind during this study.
2. Confidentiality, all patients' data will be confidential and stored in a secure location.
3. Informed consent, an informed consent will be taken from all patients and included.
4. Other ethical consideration, The research will be conducted only by scientifically qualified and trained personnel.

Administrative design:

A. Approvals obtained for performing the study from official or governmental department.

B. Approvals obtained for performing the study from Ethical committee in the faculty of medicine and from patients included in the study.

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 & median (interquartile range), and qualitative data were expressed as absolute frequencies (number)& 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 or Fisher's exact test when appropriate. Validity was calculated for both calprotein and creatinine to detect their ability to predict AKI**.** p-value< 0.05 was considered statistically significant (S), p-value ≥ 0.05 was considered statistically insignificant (NS).

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 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 odema, (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 (1)**

eISSN 1303-515

About(39%) of cases diagnosed with AKI.**As shown in figure (1)**

There were statistically significant differences between the studied groups as regard CRP as the group with AKI shows higher mean CRP 101.21±80.63.also there were statistically significant differences between the studied groups as regard TLC and platelets. While there were no statistically significant differences between the studied groups as regard HB.there were statistically significant differences between the studied groups as regard ALT, AST and INR where higher mean value was in group with AKI, also that there were statistically significant differences between the studied groups as regard albumin with lower mean value in group with AKI, While there were no statistically significant differences between the studied groups as regard albumin, AST, INR, Na, K, Ca, Phosphorus and Mg**table (2)**.

This table shows that there were statistically significant differences between the studied groups as regard Creatinine on the third day of admission where the higher mean values were cases with AKI**table (3)**.

As shown in this table regarding urinary calprotectin on first day of admission, the medianwas 214 (60-505). Regarding urinary calprotectin on third day of admission, the medianwas 416.5 (88-1267). There was statistically significant increase in calprotein level when comparing first and third Day levels p value less than 0.001**table (4)**

As shown in this table (12.8%) of AKI patient need dialysis **table (5)**.

This table shows that there were statistically significant differences between the studied groups as regard U. calprotectin on the first day of admission where the higher mean values were in cases with AKI, while there were no statistically significant differences between the studied groups as regard Creatinine on the first day of admission **table (6)**

Etiologies of intrinsic-AKI comparing their mean Calprotectin , serum Creatinine levels at the first and the third day of admission **as showed in table (7)**

Regarding validity of U. calprotectin, the value of Sensitivity was (76.9%), specificity= (76.9%), predictive value for positive (PVP) = (81.1%), predictive value for negative (PVN) = (81.1%), and (79.5%) accuracy **table (8)& figure (2).**

eISSN 1303-515

Part I

Table (1): 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±8.82 11 (6-16.75)	
Height (cm) Mean ±SD Range		89.93±23.87 (60-155)	
GCS Mean ±SD range		12.58±2.34 (6-15)	
Blood pressure 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±10.89 32 (23.25-40)	
Temperature© Mean ±SD range		37.7±3.68 (37-38)	
O2 saturation Mean ±SD range		87.51±8.74 (65-99)	
Clinical presentation	pallor	3	3
	cyanosis	45	45
	odema	46	46
Use of MV	No	48	48
	Yes	52	52
Use of nephrotoxic agent	No	55	55
	Yes	45	45

eISSN 1303-515

NB:Diagnosis of AKI in PICU based on KDIGOstaging ofAKI3

Stage	Serumcreatinine	Urineoutput
1	Increaseby1.5–1.9timesbaselinewithin7daysOR Increaseby≥0.3mg/dL(26.5µmol/L)within48hours	Lessthan0.5mL/kg/hfor6–12 hours
2	Increaseby2–2.9timesbaseline	Lessthan0.5mL/kg/hfor≥12 hours
3	Increase by ≥3 times baselineOR Increase to ≥4 mg/dL (353.6 µmol/L)OR Renal replacement therapy initiationOR Inpatientsyoungerthan18years,decreaseinestimatedGFR to <35 mL/min/1.73 m^2	Lessthan 0.3 mL/kg/hfor ≥24 hoursOR Anuriafor≥12hours

Abbreviations: KDIGO, Kidney Disease: Improving Global Outcomes;AKI,acutekidneyinjury;GFR,glomerularfiltration rate.

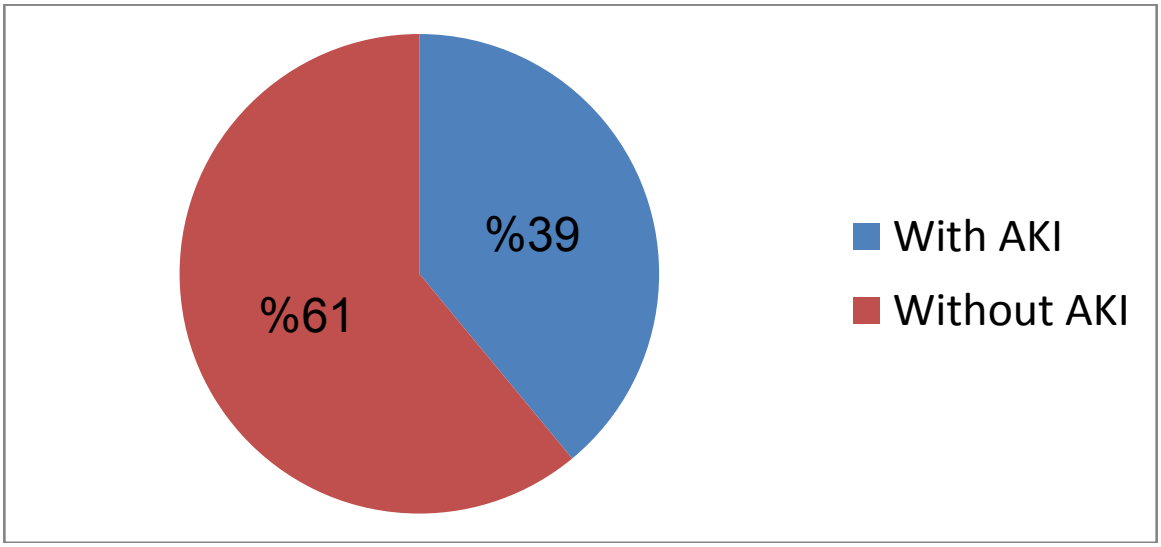

Figure (1): incidence of development of AKI within the studied groups within 48 h of PICU admission :

Table (2): relation between development of AKI and lab data of the studied group

Variables	Group without AKI (n=61)	Group with AKI (n=39)	Tests	
			t/z	P value
TLC (n×10°3) Mean ±SD Median (IQR)	16.98±36.62 11.25 (8-15)	22.71±42.78 16 (11.9-22)	-2.106	0.007*
HB(gm/dl) Mean ±SD	10.15±1.57	9.74±1.73	0.031	0.218
Platelet (n×10°3) Mean ±SD Median (IQR)	262.62±132.37 235.5 (180-375)	211.38±142.22 169 (110-299)	-2.106	0.035*
CRP(mg/l) Mean ±SD Median (IQR)	54.29±64.01 50 (7-74.75)	101.21±80.63 78 (30-168)	-3.248	0.001*
Albumin(gm/dl) Mean ±SD Median (IQR)	5.09±13.41 3.4 (3-4)	3.02±0.67 3 (2.5-3.5)	-2.878	**0.004***
ALT(u/L) Mean ±SD Median (IQR)	30.89±23.09 23.5 (15-39.75)	52.94±36.09 39 (24.8-84)	-3.207	**0.001***
AST(u/L) Mean ±SD Median (IQR)	43.02±39.14 30.5 (20-54.5)	62.61±41.31 55 (25-90)	-2.641	**0.008***
INR Mean ±SD Median (IQR)	1.14±1.04 1 (0.9-1.1)	1.15±0.31 1.2 (0.9-1.5)	-2.375	**0.018***
Na(mmol/L) Mean ±SD	137.02±8.53	133.79±6.32	2.028	0.045
K(mmol/L) Mean ±SD Median (IQR)	4.65±5.06 4 (3.5-4.5)	3.99±0.83 4 (3.3-4.6)	-0.103	0.918
Ca(mg/dL) Mean ±SD	8.75±1.27	8.47±0.98	1.159	0.249
Phosphorus(mg/dL) Mean ±SD Median (IQR)	3.82±1.07 3.95 (3.05-4.27)	4.1±2.51 3.8 (3-4.2)	-0.263	0.793
Mg (mg/dL) Mean ±SD	2.39±0.69	2.37±0.81	0.124	0.902

eISSN 1303-515

Table (3): relation between AKI and kidney function of the studied group on first and third day of admission

Variables		Group without AKI (n=61)	Group with AKI (n=39)	Test	
		Mean ±SD	Mean ±SD	*t*	P value
On first day of admission	Creatinine (mg/dL)	0.43±0.30	0.46±0.21	0.740	0.557
On third day of admission	Creatinine (mg/dL)	0.34±0.16	1.26±0.37	29.032	<0.001*

Table (4): urinary calprotectin versus serum creatinie of the AKI group on first and third day of admission

Variables	Urinary Calprotectin Median (IQR)	Serum.creat. (mg/dL) Mean ±SD
On first day of admission	214 (60-505)	0.46±0.21
On third day of admission	416.5 (88-1267)	1.26±0.37
P value	<0.001	**<0.001**

Table (5): patients' Need of dialysis within the AKI group:

Variables		AKI group (n=39)	
		No.	(%)
Need of dialysis	No	34	87.2
	Yes	5	12,8

Part II

Table (6): kidney function and urinary calprotectin of the control and AKI groups on first of admission

Variables		Group with AKI (n=39)	Control Group (n=39)	Test	
				t	P value
On first day of admission	Creatinine (mg/dL) Mean ±SD	0.46±0.21	0.39±0.13	1.854	0.068
	U. calprotectin Median (IQR)	214 (60-505)	52 (39.9-55)	-4.260	<0.001*

Table (7): Etiologies of intrinsic-AKI comparing their mean Calprotectin , serum Creatinine levels at the first and the third day of admission:

Variables		Hypovolemic shock N=7	Septic shock N=18	Cardiogenic shock N=6	Encephalitis N=5	DKA N=3
On first day of admission	Creatinine (mg/dL) Mean ±SD	0.39±0.25	0.46±0.21	0.37±0.15	0.61±0.19	0.65±0.05
	U. calprotectin Median (IQR)	76 (36.5-812)	300.5 (42.25-501.25)	69.5 (53.3-271.9)	500 (131-1016)	339 (198-567)
On third day of admission	Creatinine (mg/dL) Mean ±SD	1.18±0.37	1.31±0.43	1.03±0.09	1.29±0.23	1.53±0.35
	U. calprotectin Median (IQR)	120 (32-1228)	741 (181.25-1367.25)	81.5 (60-611.25)	564 (319-2026)	582 (132-1365)

Table (8): Validity of U. calprotectinreported

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

eISSN 1303-515

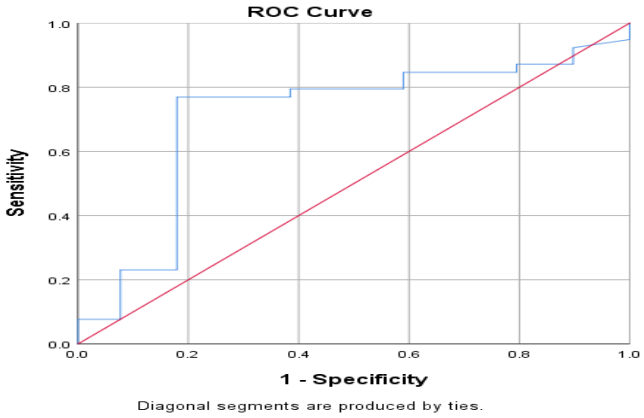

Figure (2): ROC curve for Validity of U. calprotectin

Discussion

Fecal calprotectinlevel differentiate between inflammatory bowel disease from functional intestinal problems as this marker is raised only in inflammatory intestinal problems. Therefore, we planned to use urinary calprotectin in comparison with serum BUN and creatinine and GFR to detect its sensitivity and specificity in detecting early stages of AKI in children admitted in our PICU unit. This study shows that urinary calprotectin can be a valuable biomarker to find early renal tissue damage. According to our study, urinary calprotectin level was meaningfully elevated early in children with intrinsic AKI whereas the creatinine level was not notably elevated at the same time (in the first or second day of the disease). According to our study, more than 98% of patients diagnosed as having AKI based on GFR values had elevated calprotectin. It is 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 has the highest point of sensitivity and specificity in our study. Overall, 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 [(5)].

In this study, 39% of our cases diagnosed with AKI.

This agreed with **Abdulsamea et al.** [(6)] who reported that the incidence of AKI in this study was (33%)

Al-jboor et al., [(7)] conducted study to estimate the incidence and the mortality rate of AKI in critically ill children as well as to describe some other related factors. They found of the 372 patients admitted to PICU, 64 (17.2%) patients developed AKI.

In this study, (35%) of cases diagnosed asseptic shock, (15%) diagnosed with cardiogenic shock where (14%) of them had congenital heart disease, (13%) diagnosed with respiratory failure where (12%) diagnosed with Broncho-pneumonia, (11%) of cases diagnosed with Status epilepticus, (10%) diagnosed with encephalitis, (8%) diagnosed of hypovolemic shock, (3%) diagnosed of DKA. Each of tumor lysis syndrome, meningitis, co poisoning, SHA were diagnosed with (1%).

This is in harmony with **Ghobrial et al.** [(8)] who reported that, sixteen out of 90 patients (17.7%) included in the study suffered from congenital heart disease (CHD). This may be due to the late presentation of cases as we are tertiary care center and many patients with CHD are referred to us. Neonates with CHD who undergo cardiac surgery are vulnerable to AKI.

The current study showed that, regarding urinary calprotectin on first day of admission, and the median was 214 (60-505). Regarding urinary calprotectin on third day of admission, the median was 416.5 (88-1267). There was statistically significant increase in calprotein level when comparing first and third Day levels p value less than 0.001.

This is in harmony with**Vakili et al** [(1)] who reported that, urinary calprotectin level was meaningfully elevated early in children with intrinsic AKI whereas the creatinine level was

eISSN 1303-515

not notably elevated at the same time (in the first or second day of the disease).

Calprotectin is an immunomodulatory protein, regarded as an inflammatory factor, and has a protective role in oxidative processes of inflammation [(9)].

The urinary calprotectin is higher in intrinsic kidney injury than prerenal kidney injury. It may be reasonable to conclude that urinary calprotectin is a good diagnostic test in the discrimination of an intrinsic kidney injury with a pooled diagnostic accuracy of symmetric SROC of 0.9667. It has been noted in earlier studies that calprotectin is released from the immune system cells (neutrophils and to lesser degree monocytes) and renal collecting duct epithelial cells [(10)].

It has also been demonstrated that renal tubular epithelial cells produce calprotectin in response to unilateral ureteral obstruction [(11)].

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

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 [(5)].Their study showed that urinary calprotectin has a good performance by a cut-off value of 230 ng/mL, in differentiation of structural and functional AKI in pediatric population with high sensitivity (95.6%) and specificity (100%).

Heller *et al*. [(12)] performed such a study and resulted that calprotectin has a high diagnostic value in intrinsic AKI and at a 300 ng/mL level has 92.3% sensitivity and 97.1% specificity.

Heller et al. [(12)] showed high accuracy of urinary calprotectin for diagnosis of intrinsic AKI. They demonstrated that the cut-off level of 300 ng/mL has a sensitivity of 92.3% and specificity of 97.1% in predicting intrinsic AKI.

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.

References:

1. **Vakili M, Fahimi D, Esfahani ST, Sharifzadeh M, Moghtaderi M.** Comparative Analysis between Urinary Calprotectin and Serum Creatinine for Early Detection of Intrinsic Acute Kidney Injury. Indian J Nephrol. 2021 Jul-Aug;31(4):353-357.
2. **Seibert FS, Pagonas N, Arndt R, Heller F, Dragun D, Persson P, et al.**Calprotectin and neutrophil gelatinase-associated lipocalin in the differentiation of pre-renal and intrinsic acute kidney injury. ActaPhysiol (Oxf) 2013;207:700–8.
3. **Westhoff JH, Seibert FS, Waldherr S, Bauer F, Tonshoff B, Fichtner A, et al.** Urinary calprotectin, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin for the prediction of adverse outcome in pediatric acute kidney injury. Eur J Pediatr. 2017;176:745–55.
4. **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. PediatrNephrol. 2016;31:2353–63.
5. **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. Iran J Pediatr. 2017;27:e9727.
6. **Abdulsamea SZ, AbdulHalim AS, Abed NT, AbdElhady S**. Acute Kidney Injury (Aki) In Critically Ill Children: Incidence, Diagnosis & Outcome Among Patients Of One Of Egyptian Districts (Benha University Hospital). Al-AzharAssiut Medical Journal. 2015 Jul;13(3).
7. **Al-jboor W, Almardini R, Al Bderat J, Frehat M, Al Masri H, Alajloni MS**. Acute kidney injury in critically ill child. Saudi J Kidney Dis Transpl 2016;27:740-7
8. **Ghobrial EE, Elhouchi SZ, Eltatawy SS, Beshara LO**. Risk factors associated with acute kidney injury in newborns. Saudi J Kidney Dis Transpl 2018;29:81-7
9. **Seibert FS, Rosenberger C, Mathia S, Arndt R, Arns W, Andrea H, Pagonas**

N,Bauer F, Zidek W, Westhoff TH: Urinary calprotectin differentiates between prerenal and intrinsic acute renal allograft failure. Transplantation 2017; 101: 387-394.

10. **Ebbing, J.; Seibert, F.S.; Pagonas, N.; Bauer, F.; Miller, K.; Kempkensteffen, C.; Gunzel, K.; Bachmann, A.; Seifert, H.H.; Rentsch, C.A.; et al.** Dynamics of Urinary Calprotectin after Renal Ischaemia. PLoS One 2016, 11, e0146395.
11. **Fujiu, K.; Manabe, I.; Nagai, R.** Renal collecting duct epithelial cells regulate inflammation in tubulointerstitial damage in mice. J. Clin. Investig. 2011, 121, 3425–3441.
12. **Heller F, Frischmann S, Grünbaum M, Zidek W, Westhoff TH**. Urinary calprotectin and the distinction between pre-renal and intrinsic acute kidney injury. Clin J Am SocNephrol. 2011;6:2347–55.