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Heat Shock Protein-72 as a Novel Biomarker to Predict Acute Kidney Injury in Critically Ill Patients

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ABSTRACT

Background: Acute kidney injury (AKI), also known as transient renal failure, is characterized by a sudden loss in kidney function and a reversible acute rise in nitrogen waste products. A measurable decrease in urine output can be used to identify renal function decline. Blood tests for the kidney's typical excretion products, urea and creatinine, are frequently used to identify it. AKI in rats and people can be detected early by using a sensitive biomarker called heat shock protein-72 (Hsp 72). The biomarker Hsp-72 has sufficient sensitivity and specificity to identify the AKI up to 3 days before the diagnosis in severely sicked individuals.

Aim: This study aimed to assess the sensitivity, specificity, and predictive values of Hsp-72 in the early prediction of AKI in severely diseased candidates.

Subject and Methods: This study was done in PICU, Pediatrics Department, Zagazig University. Participants were split into 2 collections according to development of AKI.

Results: There was significance elevation in neutrophil gelatinase-associated lipocalin (NGAL), KIM-1 and Hsp-72 levels at 3rd day compared to levels on admission. Urinary output showed significant decrease in AKI group compared to no AKI group. There were no statistical significance variations among the double collections as regards nephrotoxic drug. **Conclusion:** Even though most of the investigated biomarkers had comparable capacities to inspect the AKI 24-h before the AKIN features was met, Hsp-72 was much utmost due to it was the initial discoverable AKI biomarker and was remarkably sensitive and specific. HSP-72 was the most popular diagnosis at day 3. AKI in severely sicked individuals can be accurately and specifically predicted by the biomarker HSP-72 up to three days before the diagnosis.

Keywords: Heat shock protein-72, Acute kidney injury, Pediatric AKI.

INTRODUCTION

Acute kidney injury (AKI), also known as acute renal failure (ARF), is a medical disorder that manifests as an hour-to-week-long reversible acute rise in nitrogen waste products as evaluated by serum creatinine and blood urea nitrogen (BUN) [(1)].About 5% of kids admitted to paediatric intensive care units and those undergoing cardiac procedure for congenital cardiac disease experience acute renal damage. The showed incidence between 30 to 40%, and in children receiving bone marrow transplantation the incidence ranges from 15 to 34% [(2)].

Heat shock protein-72 (Hsp 72) has been demonstrated by **Sanchez-Pozos *et al.*** [(3)] to be an earlier and specific biomarker for AKI in mice and individuals. Additionally, this original biomarker was useful at observing a reno-protective approach in an investigational mice sort of AKI as well as stratifying various levels of tubular injury and recovery.

PATIENTS AND METHODS

This study was a prospective cohort research made in PICU, Department of Pediatrics, Zagazig University Hospitals in 6 months from October to March 2022.

Sample size:

Assuming that the total population size of children in Pediatric Intensive Care Unit (PICU) in 6 months duration is 160 patients and the positive predictive value of Hsp-72 in detection of AKI is 95%. So, the sample size was 50 patients using open Epi program with CI95%.

Study population:

This study included 50 critically ill patients who were admitted to PICU exhibiting two or more organ failures and were investigated at day 1, 3 according to the increase of serum creatinine and decrease urinary output. Candidates were split to double collections:

- **AKI group:** included 21 cases.
- **No AKI group:** included 29 cases.

Inclusion criteria:

Children attended to the pediatric intensive care unit who had at least 2 or more dysfunctional organs. With mechanical ventilation, one of the organ failures is the glomerular filtration rate (GFR), which is studied by means of the Schwartz equation:

$$eGFR = k \times \frac{\textbf{height in cm}}{\textbf{serum creatinine}}$$

It is recommended to use presumed baseline of 120 ml/minute/1.73 m^2, with no AKI at the time of admission.

Exclusion criteria:

Patients with chronic kidney disease under renal replacement therapy (patients on renal dialysis were excluded) or under conservative treatment.

Acute Kidney Injury: AKI is defined as a rise in serum creatinine of 0.3 mg/dl within 48 hours or a rise of 50% from the level at admission while hospitalised

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(KIDIGO. 2012) and a reduction in urination production as Half ml/kg/h or more (within 6- 12hrs).

Operational design: All patients involved in this study were submitted to the following:
- Complete history taking through a standardized clinical sheet **(**Name, age, sex, cause of admission, consanguinity and history of nephrotoxic drugs administration).
- General examination **(**Blood pressure measurement, heart rate and respiratory assessment on ventilation) and local examination (C.N.S, Heart, Chest and Abdomen).

- Laboratory investigations:

Complete blood count (CBC) was done using **xs-500i-cell counter (sysmex)**. Serum Na and K were done using **Cobas 80000 Autoanalyser (Roch Diagnostics).** Blood urea and serum creatinine were done using **Cobas 80000 Autoanalyzer (Roch Diagnostics),** Creatinine clearance and Urinary HSP-72 was measured at day 1 and day 3 by a research enzyme-linked immunosorbent assay (ELISA).

Ethical Approval:

Each participant in the study provided written informed consent, which was obtained after the study was granted permission by Zagazig University's Ethics Committee. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduction of this work.

Statistical analysis

Statistical package for social sciences (SPSS) version 26.0, Microsoft Excel 2016, and MedCalC programme software version 19.1 were used to tabulate and statistically analyse the collected data. For quantitative variables, inferential analyses were performed using the independent t-test for children of two independent groups with parametric data and the Mann Whitney U for children of two independent groups with non-parametric data.

The Chi square test for independent groups was used for inferential analysis of qualitative data. The ROC curve (receiver operating characteristic) is a valuable tool for assessing the sensitivity and specificity of quantitative diagnostic measures that classify cases into one of two categories. P values ≤ 0.05 were used to determine the significance level; values beyond this threshold are non-significant. A statistical metric for the probability that the results observed in a study could have occurred by chance.

RESULTS

This prospective cohort study was conducted on 50 seriously diseased kids came to Pediatric Intensive Care Unit, Department of pediatrics, Zagazig University Hospitals with no AKI at the time of admission.

Fifty critically ill children were included in our study. The age of patients ranged from 3 to 10 years with mean age of 5.48 ± 2.13 years. There were 27 males and 23 females with male to female ratio was 1.17:1. Regarding residence, 58% of patients were living in rural areas and 42% of patients were living in urban areas. 11 patients (22%) had positive consanguinity (Table 1).

Table (1): Distribution of studied children as per demographic characteristics

Parameters		Studied children (n= 50)	
		N	%
Age (years)	Mean± SD	5.48± 2.13	
	Median	5.0	
	Range	3.0 – 10.0	
Sex	Male	27	54.0%
	Female	23	46.0%
Residence	Rural	29	58.0%
	Urban	21	42.0%
Consanguinity	No	39	78.0%
	Yes	11	22.0%

SD= standard deviation, n: number, %: percentage

The chief public reason of hospital entry in the studied patients was respiratory disease and septicemia representing 22% followed by multiple organ dysfunction syndrome and neurologic disease in 16% patient's then cardiac disease and gastroenterological disease in 12% patients (Table 2).

Table (2): Distribution of studied children as per cause of admission

Parameters		Studied children (n= 50)	
		N	%
Cause of admission	Cardiac disease	6	12.0%
	Gastroenterological disease	6	12.0%
	MODS	8	16.0%
	Neurologic disease	8	16.0%
	Respiratory disease	11	22.0%
	Septicemia	11	22.0%

MODS: Multiple Organ Dysfunction Syndrome, n: number, %: percentage.

42% of patients were treated by inotropic drugs and 46% of patients had history of taking nephrotoxic drug (Table 3).

Table (3): Distribution of studied children as per inotropic drugs and nephrotoxic drugs

Parameters		Studied children (n= 50)	
		N	%
Inotropic drugs	No	29	58.0%
	Yes	21	42.0%
Nephrotoxic drug	No	27	54.0%
	Yes	23	46.0%

SD= standard deviation, n: number, %: percentage

The mean Hsp-72 on admission and at 3rd day was 0.38 ± 0.03 and 3.56 ± 3.84 respectively. There was significant elevation in Hsp-72 levels at 3rd day compared to levels on admission (p<0.001). Also, there was significant elevation in KIM-1 at 3rd day when compared to that of at admission in AKI group (p<0.001) **(Table 4).**

Table (4): Hsp-72 among the studied children on admission and after 3rd day

		On admission	At 3rd day	*Wilcoxon Signed Ranks Test*	
				Z	P-value
NGAL	Mean± SD	17.0 ± 4.23	34.34 ± 21.31	4.448	**<0.001****
	Median	17.0	21.0		
	Range	11.0- 25.0	9.0- 73.0		
KIM-1	Mean± SD	19.44 ± 3.85	38.60 ± 23.01	4.007	**<0.001****
	Median	19.0	25.0		
	Range	13.0- 28.0	13.0- 79.0		
Hsp-72	Mean± SD	0.38 ± 0.03	3.56 ± 3.84	3.535	**<0.001****
	Median	0.38	0.41		
	Range	0.32- 9.5	0.21- 9.5		

P value < 0.05 is significant, P value < 0.01 is highly significant, SD: Standard deviation.

The studied children were divided into two groups according to AKI progression. AKI is described like a 50% elevation of serum creatinine from the level at admission to the hospital or a rise in serum creatinine of 0.3 mg/dl on the third day. 21 patients with AKI made up Group A. 29 individuals in Group B who had no AKI. There was no significant difference between the two groups regarding Hsp-72 at admission while there Hsp-72 was significantly higher in AKI group compared to non-AKI group at 3rd day (p<0.001). While, in no AKI group there was no significant difference (Table 5).

Table (5): Comparison between the studied groups regarding Hsp-72 on admission and at 3rd day

Hsp-72 (ng/mL)		AKI group (No. = 21)	No AKI group (No. = 29)	*Mann- Whitney U test*	
				ZMWU	P-value
On admission	Mean± SD	0.38 ± 0.38	0.38 ± 0.38	0.138	0.890
	Median	0.38	0.38		
	Range	(0.33- 0.46)	(0.32- 0.46)		
After at 3rd day	Mean± SD	7.9 ± 0.95	0.36 ± 0.04	5.99	**<0.001**
	Median	7.9	0.37		
	Range	(5.8- 9.5)	(0.21- 0.46)		
Wilcoxon Signed Ranks Test	Z	4.02	1.31		
	P-value	**<0.001**	0.189		

P value< 0.05 is significant, P value< 0.01 is highly significant, SD: Standard deviation.

NGAL, KIM-1 and Hsp72 at 3rd day were good predictors for AKI in children with area under the curve 0.99. NGAL during the third day can predict AKI with 90% and 95% specificity and sensitivity respectively (p< 0.001). KIM-1 can predict AKI with 90% sensitivity and 98% specificity on the third day (<p 0.001). Hsp-72 can predict AKI with 92% and 94% sensitivity and specificity on the third day, respectively (p< 0.001) (Figure 1).

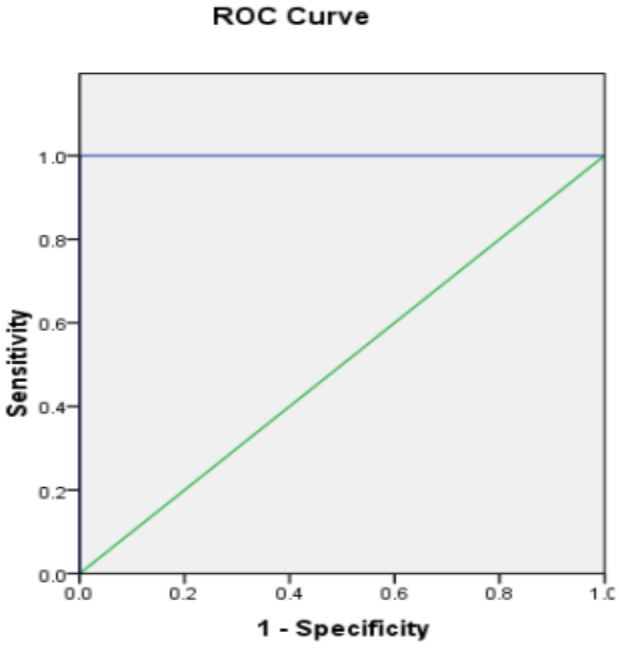

Figure (1): Roc curve of Hsp-72 at 3rd day.

DISCUSSION

Acute kidney damage (AKI) is a prevalent illness that worsens mortality and morbidity in the critically unwell. Currently, a functional diagnosis of AKI is obtained based on uprising in the plasma creatinine and/or a reduction in urine output. Both of these are not optimal, hence the development of new, accurate, and early kidney damage indicators is necessary [(4)].

This prospective cohort study was conducted on 50 seriously diseased kids came to Pediatric Intensive Care Unit, Department of Pediatrics, Zagazig University Hospitals with no AKI at the time of registration. All participants were distributed into double collections: AKI group included 21 cases. No-AKI group included 29 cases. AKI is recognized by an elevation in serum creatinine by 0.3 mg/dl at 3rd day or a 50% uprising in serum creatinine from the level on admission during hospitalization.

Regarding demographic data of the studied cohort, we found that the candidates aged from 3 to 10 years had mean age of 5.48 ± 2.13 years. There were 27 boys and 23 girls with male to female ratio was 1.17:1. Regarding residence, 58% of patients were living in rural areas and 42% of patients were living in urban areas. 11 patients (22%) had positive consanguinity. There were no statistically significance variations between the two collections concerning age and sex (P value >0.05). Concerning residence and consanguinity, there were no remarkably significant variations among the two collections (P value >0.05).

This is in agreement with **Morales-Buenrostro *et al.*** [(5)] study aimed to identify whether the biomarkers neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), and heat shock protein (Hsp-72) are more accurate and early indicators of acute kidney injury (AKI). 56 severely ill candidates were enrolled in the trial. Twenty of these patients were chosen as controls, and 17 of them suffered from AKI. The study found no discernible difference in the studied groups' ages or sexes. Also, **Rustagi *et al.*** [(6)] reported that there were no remarkably significance variations among the AKI and No-AKI pediatric collections regarding age and sex.

Distribution of studied children as per inotropic drugs and nephrotoxic drugs showed that 42% of patients were treated by inotropic drugs and 46% of patients had history of taking nephrotoxic drug. History of taking ionotropic drugs was significantly higher in AKI group compared to no-AKI group (p=0.003). Meanwhile, there were no statistically significant differences between the two groups regarding nephrotoxic drug (p-value >0.05). This comes in agreement with **De Zan *et al.*** [(7)] who reported that the hazard of AKI was definitely linked with the amount of inotropes in critically ill children.

Also, the study by **Aygun *et al.*** [(8)] reported that inotropic drugs use was significantly associated with AKI in critically ill children.

Regarding Hsp-72 among the studied children on admission and after 3rd day, we found that the mean Hsp-72 on admission and at 3rd day was 0.38 ± 0.03 and 3.56 ± 3.84 respectively. There was significant elevation in Hsp-72 levels at 3rd day compared to levels on admission (p<0.001). The current results are supported by study of **Morales-Buenrostro *et al.*** [(5)] whereas they reported that from two days earlier to the diagnosis of AKI, the levels of KIM-1, IL-18, NGAL, and Hsp-72 rapidly increased and remained elevated throughout. Additionally, they showed that urine Hsp-72 levels rose in critically ill patients beginning 3 days before the diagnosis of AKI. This early rise in levels was not seen with any other investigated biomarkers.

The current study also revealed that Hsp-72 levels were similar in both groups at admission but considerably greater in the AKI group compared to the non-AKI group on day three (p<0.001).

The current results are supported by study of **Morales-Buenrostro *et al.*** [(5)] who showed that over their 10 days in the ICU, candidates with no-AKI had urine Hsp-72 measurements of about 0.3 ng/ml, which remained unaltered. On the other hand, patients with AKI had significantly higher urine Hsp-72 levels 3 days prior to the AKI being identified via the AKIN features (p = 0.045).

Also, **Barrera-Chimal *et al.*** [(9)] reported that urine Hsp-72 levels steadily raised in association with the degree of renal damage caused by various ischemia times, as measured by histomorphometry as a reference of renal damage. According to histopathological findings, urinary Hsp-72 started to return to normal after 3 hours and continued to rise until 18 hours later. Normalization of urine Hsp-72 levels was linked to spirolactone renoprotection. Therefore, before the elevation of serum creatinine, urine Hsp-72 was considerably higher in patients with clinical AKI.

Our results showed that the best diagnostic preference was found in the use of Hsp-72 at 3rd day. This is in agreement with **Morales-Buenrostro *et al.*** [(5)] who found that KIM-1 and Hsp-72 showed the highest sensitivity and specificity, with values of 83/95% and 100/90%, respectively, compared to 100/100% and 100/90%, respectively, 1 day prior to the diagnosis of AKI. The validation test for Hsp-72 had 100% sensitivities, 83.3% specificities, and 90.9% accuracy, respectively. According to the study's findings, the biomarker Hsp-72 can detect AKI in severely diseased participants up to three days before the diagnosis by being sufficiently sensitive and specific.

CONCLUSION

Although the majority of the evaluated biomarkers showed a comparable capacity to expect AKI, 24-hours before the AKIN measures were met, Hsp-72 was unquestionably a more sensitive and specific AKI biomarker. Because the majority of AKI cases was categorised as AKIN 1. A class that clinicians believed to be of little clinical importance or influence.

The cost-benefit analysis would support the daily urine of Hsp-72 recognition, starting at ICU entry and continuing for three to five days after ICU discharging, in order to effectively diagnose AKI before conventional markers. On the third day, NGAL, KIM, and HSP-72 were accurate biomarkers for spotting AKI in seriously unwell kids. The diagnostic biomarker HSP-72 exhibited the highest sensitivity on day 3, and it was sensitive and specific to expect the AKI in severely diseased participants up to three days before the identification.

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