The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89, Page 4197- 4204

Proenkephalin A119-159 As A Biomarker of Acute Kidney Injury in ICU Patients with Sepsis

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ABSTRACT

Background: Sepsis is a potentially fatal illness caused by an unbalanced host response to infection. Sepsis is becoming more common, indicating that it is a main cause of severe disease and fatality globally. Patients with sepsis commonly have simultaneous acute kidney injury (AKI), with rates ranging from 5 to 20% among hospital admissions and increasing to 35-50% among the seriously sick. Because proenkephalin is not bound to proteins in plasma and is only filtrated in the glomerulus, it is a promising biomarker for renal impairment in critically sick patients. **Objective:** The aim of the current study was to assess Proenkephalin A119-159 (penKid) as a biomarker for sepsis related AKI in ICU patients. **Patients and methods:** A case control study was conducted at intensive care unit (ICU) department, Benha Faculty of Medicine on a total of 80 subjects which were divided into 50 septic AKI cases and 30 matched age and sex controls. Subjects met two or more SIRS-criteria and their estimated glomerular filtration rate (eGFR) was determined by the formula derived from the Modification of Diet in Renal Disease (MDRD) Study and the Proenkephalin A119-159 (penkid) was measured by ELISA. **Results:** The current study demonstrated that, septic AKI cases demonstrated significant increase in penkid compared to control group($p<0.001$) and Penkid level showed significantly positive correlation with baseline, after 48 h creatinine, CRP, SIRS and SOFA scores. Penkid was better than creatinine, eGFR and CRP for prediction of non-recovery septic AKI. **Conclusion:** PenKid was demonstrated to be a reliable surrogate promising biomarker for sepsis related AKI among unselected patients with sepsis. Additionally, penkid demonstrated superior advantage over creatinine, eGFR and CRP in terms of non-recovery septic AKI prediction.

Keywords: Sepsis, Systemic Inflammatory Response Syndrome, Proenkephalin, acute kidney injury, Benha University.

INTRODUCTION

Kidney Disease Improving Global Outcome (KDIGO) defines acute kidney injury (AKI) as one of the following: a rise in serum creatinine of 0.3 mg/dL or more within 48 hours; a rise in serum creatinine of 1.5 times baseline or more within the last 7 days; or a decrease in urine output of less than 0.5 mL/kg/h for a period of six hours [1]. While, because existing standard biomarkers lack sufficient sensitivity or specificity, its early diagnosis remains difficult [2].

The fact that serum creatinine might not rise despite renal damage in sepsis-related AKI may be a significant issue. This could be brought on by a reduction in the synthesis of serum creatinine or its dilution as a result of IV fluid delivery. It could also be brought on by serum creatinine's unfavourable kinetics, which does not increase for 24 to 48 hours following renal damage [3]. Implementing innovative biomarkers that allow for a trustworthy classification of AKI risk for ICU patients will allow for the early development of effective management regimens with a potential benefit to patient outcomes [4]. With regard to predicting AKI, the use of biomarkers for kidney damage or injury has shown mixed results, mostly because kidney damage and loss of renal function are not correlated with one another [5].

Proenkephalin A119-159 (penkid), a filtration marker, has recently been suggested as a sensitive biomarker of glomerular function. Penkid, a 5-kDa peptide thought to be a stable surrogate marker for the unstable enkephalins, is generated from the same precursor as met- and leu-enkephalins [6].

There is expression of proenkephalin A119-159 (penkid) in a variety of tissues, including the kidney and the heart. Penkid is regarded as a marker of kidney function (not injury) because of the substantial negative correlation between observed glomerular filtration rate (GFR) and plasma concentrations of penkid [7].

Penkid levels rise faster than creatinine levels do in the presence of acute renal failure. In contrast to other indicators, an increase in plasma penkid does not appear to be affected by factors unrelated to renal function, such as systemic inflammation, suggesting that it is a highly specific marker for kidney disease [8].

The aim of the current study work was to assess penKid as a biomarker for sepsis related AKI among unselected patients with sepsis.

PATIENTS AND METHODS

This study was conducted at intensive care unit (ICU) department, Benha Faculty of Medicine on a total of 50 patients with 30 matched age and sex controls.

Ethical approval:

After obtaining the Approval from Ethical Committee of Benha Faculty of Medicine. All participants signed an informed consent before being included 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.

We included patients who were 18 years and older, who admitted to the ICU for sepsis or septic shock, whom had two or more SIRS-criteria and we excluded subjects

Received: 26/4/2022
Accepted: 23/6/2022

under 18years of age, pregnant ladies and whom had vegetative coma.

Entire subjects were divided into 2 group: Group (I) [case group]: including 50 subjects with suspected infection, and meeting of two or more SIRS-criteria (Systemic Inflammatory Response Syndrome) [9], **and Group ((II) [control group]:** including 30 apparently healthy subjects.

SIRS is defined as: (1) Either body temperature of more than 38°C, or less than 36°C. (2) Respiration rate of more than 20 breaths/min. (3) A heart rate more than 90 beats/min. (4) Leukocytosis >12.000/ mm^3 or leucopenia <4.000/ mm^3. (5) New altered mental state. (6) Blood glucose >7.7mmol/L but not diabetic.

All participants were subjected to full history taking, full clinical examination, thorough laboratory tests [as complete blood picture, C-reactive protein, serum creatinine, serum bilirubin, and PT-International Normalized Ratio (INR)], their Proenkephalin A119-159 (penkid) was measured by ELISA, and their estimated glomerular filtration rate (eGFR) was determined by the formula derived from the Modification of Diet in Renal Disease (MDRD) Study [10].

Statistical analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Sciences (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Student's t test was used to assess the statistical significance of the difference between two study group means. For the comparison of the three groups' means, one way analysis of variance (ANOVA) was used. Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. The Kruskal-Wallis test, it was used to assess the statistical significance of the difference between more than two study group non-parametric variables. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. The ROC Curve (receiver operating characteristic) provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. Regression analysis: Linear regression analysis was used for prediction of risk factors, using generalized linear models. Odds ratio and 95% confidence interval were calculated. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. Cox regression analysis of factors potentially related to survival was performed to identify which independent factors might jointly have a significant influence on survival. Probability: A p-value is considered significant if ≤0.05 at confidence interval 95%.

RESULTS

The present study included 50 septic AKI patients and 30 healthy control subjects with mean age of septic AKI group was 64 years, they were 20 males (40%) and 30 females (60%) (**Table 1**).

Table (1): Comparison of demographic data among studied groups.

Variable		Control N=30	Septic AKI N=50	P value
Age (years)	**Mean ± SD**	67.1 ± 17.3	64 ± 15.7	0.417
Males	**N, %**	15, 50%	20, 40%	0.383
Females	**N, %**	15, 50%	30, 60%	

SD, standard deviation; N, number.

Control group was selected to be matched in age and gender. The most common cause of sepsis was UTI (44%) followed by chest infection (40%). More than half of studied cases required mechanical ventilation (56%) with median duration of 5 days and ranged from 2 to 15 days. While, only 26% needed replacement therapy (**Table 2**).

Table (2): Causes of sepsis in septic AKI group.

Variable			**Septic AKI N=50**	
UTI		**N, %**	22	44%
Chest infection		**N, %**	20	40%
Others	**Bed sores**	**N, %**	1	2%
	Cellulites	**N, %**	1	2%
	CRBSI	**N, %**	1	2%
	gluteal abscess	**N, %**	1	2%
	Infected foot wound	**N, %**	1	2%
	infected surgical wound	**N, %**	1	2%
	Pernepheric abscess	**N, %**	1	2%
	spontaneous bacterial peritonitis	**N, %**	1	2%
Mechanical ventilation and replacement therapy	**Patient needed mechanical ventilation**	**N, %**	28	56%
	How long patient is ventilated (days)	**Median (range)**	5	2-15
	Patient needed replacement therapy	**N, %**	13	26%

Septic AKI group was significantly associated with higher penkid when compared to control group (**Table 3**).

Table (3): Comparison of Penkid among studied groups.

Variable	Control N=30	Septic AKI N=50	P-value
	Mean ± SD	Mean ± SD	
Penkid (pmol/L)	729.1±176.1	2475.6± 588.3	**<0.001**

Receiver operating characteristic (ROC) curve of penkid was conducted for discrimination between septic AKI and control groups. Penkid showed high accuracy AUC (AUC=0.985). Best cut off value was 1200.65ng/ml, sensitivity was 90%, specificity was 100%, PPV was 90%, NPV was 100%, and accuracy was 93.8% (**Table 4, Figure 1**).

Table (4): Validity of Penkid for prediction of septic AKI occurrence.

Validity Data	Penkid
AUC	0.985
Cut off	1200.65
Sensitivity (%)	90
Specificity (%)	100
PPV (%)	90
NPV (%)	100
Accuracy (%)	93.8

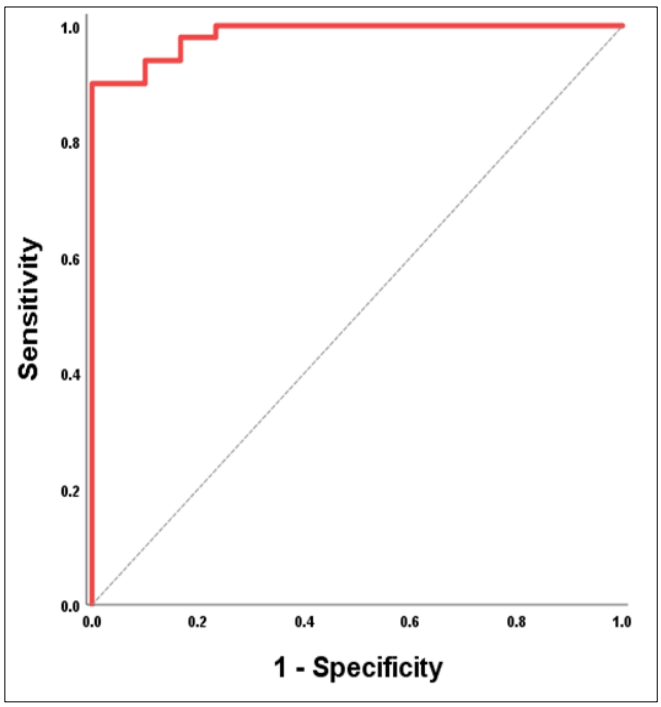

Figure (1): ROC curve of Penkid for prediction of septic AKI occurrence.

AKI cases who required replacement therapy showed higher level of penkid when compared to those who did not require (table 5, fig. 2). ROC curve of penkid was conducted for prediction of requirement of replacement therapy (fig. 3). Penkid showed high accuracy AUC (AUC=0.860), best cut off value of was 2609.4ng/ml, Performance characteristics are shown in **Table (6).**

Table (5): Comparison of Penkid level according to requirement of replacement therapy.

Variable		Requirement of replacement therapy		P-value
		No (N=37)	Yes (N=13)	
Penkid	**Mean ± SD**	2376.3± 530.6	2717.9± 557.9	**<0.001**

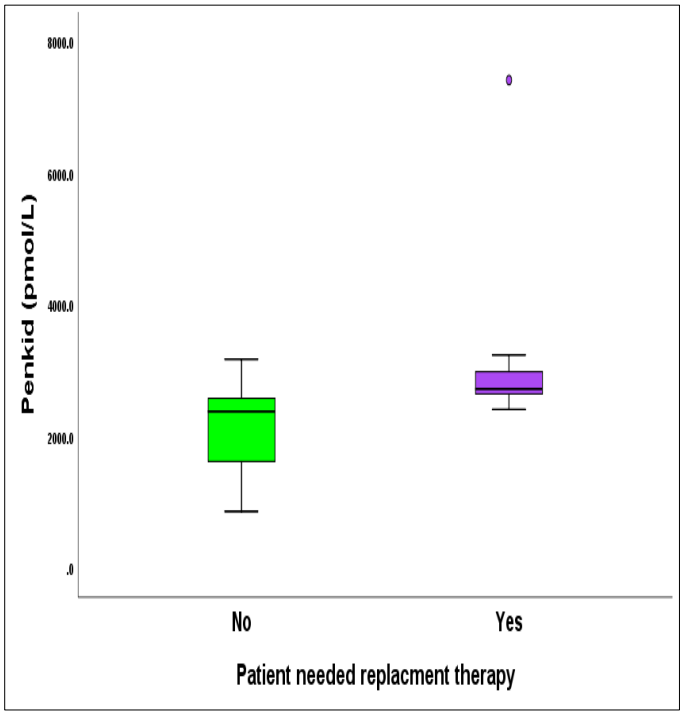

Figure (2): Penkid level according to requirement of replacement therapy.

Table (6): Validity of Penkid for prediction of requirement of replacement therapy.

Validity Data	Penkid
AUC	0.860
Cut off	2609.35
Sensitivity (%)	84.6
Specificity (%)	78.4
PPV (%)	84.6
NPV (%)	78.4
Accuracy (%)	80.0

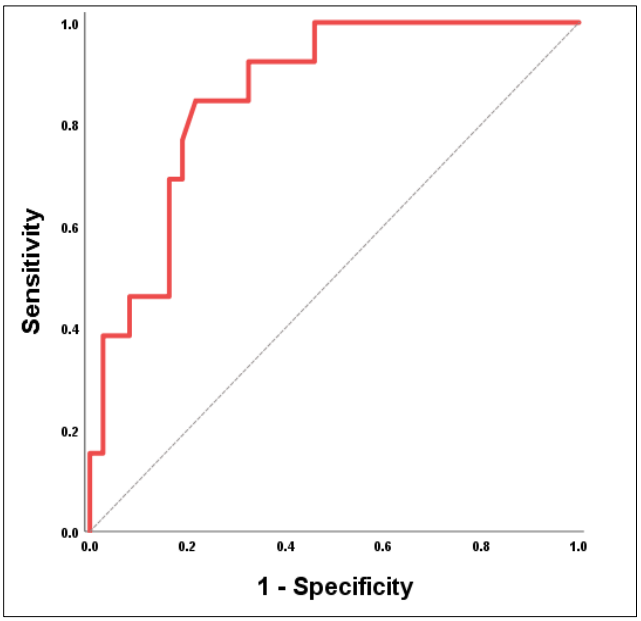

Figure (3): ROC of Penkid for prediction of requirement of replacement therapy.

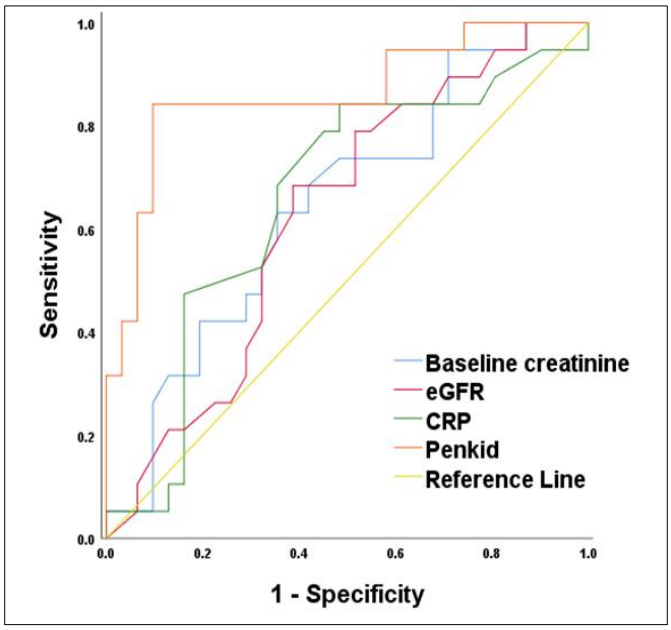

Figure (4): ROC curve of creatinine, eGFR, CRP and penkid for prediction of non-recovery outcome.

ROC curve of creatinine, eGFR, CRP and penkid was conducted for prediction of non-recovery outcome (CKD &/or death). Creatinine, eGFR, CRP showed low accuracy AUCs (AUC=0.647, 0.628, 0.660 respectively). Regarding penkid, high accuracy AUC was found (AUC=0.862), best cut off value of was 2609.4ng/ml, sensitivity was 84.2%, specificity was 90.3%, PPV was 84.2%, NPV was 90.3%, and accuracy was 88%. Penkid AUC was better than creatinine, eGFR and CRP for prediction of non-recovery septic AKI (**Table 7, Figure 4**).

Table (7): Validity of Penkid for prediction of non-recovery outcome (CKD and/or death).

Validity Data	Baseline creatinine	eGFR	CRP	Penkid
AUC	0.647	0.628	0.660	0.862
Cut off	4	<20.5	123	2609.4
Sensitivity (%)	63.2	68.4	68.4	84.2
Specificity (%)	64.5	61.3	64.5	90.3
PPV (%)	63.2	68.4	68.4	84.2
NPV (%)	64.5	61.3	64.5	90.3
Accuracy (%)	64	64	66	88

Penkid level tend to increase significantly with increased SIRS score in septic AKI cases. Higher Penkid level was significantly associated with non-recovery compared to recovery, as well as to non survivors compared to survivors. No significant differences was found between those developed CKD and those did not, this may be attributed to small sample size of CKD [n=3] (**Table 8**).

Table (8): Association of Penkid level with SIRS score and outcome in septic AKI cases.

Penkid		N	Median	Range	P-value
SIRS score	**SIRS**	1	2000.2	2000.2-2000.2	**<0.001**
	Sepsis	36	2390.1	859.1-7410.2	
	Severe sepsis	6	2904	2669.4-3169.6	
	MOD	7	2787.7	2642.3-3233.6	
Recovery			2376.3	859.1-2913.7	**<0.001**
No recovery			2729.6	1287.2-7410.2	
CKD			2491.1	859.1-7410.2	0.438
No CKD			2000.2	1287.2-2642.3	
Survivors			2279.4	(859.1-2913.7)	**<0.001**

Penkid level showed a significantly positive correlation with baseline, after 48 hours creatinine, CRP, SIRS and SOFA scores. Otherwise, no significant correlations were found between Penkid with other parameters in all studied cases (**Table 9**).

Table (9): Correlations of Penkid with other parameters in all studied cases.

Penkid	Rs	P
Age	0.149	0.301
BMI	0.225	0.121
Duration	0.166	0.227
Urea	0.206	0.131
basal creatinine	0.550	**<0.001**
after 48 h creatinine	0.914	**<0.001**
Egfr	-0.178	0.215
CRP	0.780	**<0.001**
SIRS score	0.606	**<0.001**
SOFA score	0.987	**<0.001**

Cox regression analysis was conducted for prediction of OS in septic AKI cases, using age, gender, BMI, comorbidities, eGFR, SIRS, SOFA, penkid level as confounders. Higher SIRS, SOFA, Penkid level were associated with poorer OS in unvariable analysis. Note that eGFR did not predict mortality. However, in multivariable analysis, higher SOFA, Penkid level were suggested to be independent predictors of shorter OS in septic AKI cases (**Table 10**).

Table (10): Cox regression analysis for prediction of OS in septic AKI cases.

Variable	Univariable				Multivariable			
	P	HR	95% CI		P	HR	95% CI	
Age	0.447	1.014	0.979	1.050				
Gender	0.401	0.657	0.247	1.751				
BMI	0.115	1.174	0.332	1.336				
Comorbidity	0.646	1.415	0.321	6.231				
eGFR	0.389	0.981	0.940	1.024				
SIRS	**0.001**	1.820	1.293	2.561	0.764	1.074	0.673	1.716
SOFA	**<0.001**	1.596	1.305	1.950	**0.001**	1.656	1.219	2.249
Penkid	**<0.001**	2.718	1.649	4.480	**<0.001**	1.014	1.005	1.076

DISCUSSION

A dysregulated host response to infection can result in sepsis, a potentially fatal illness. Septic shock, which is frequently accompanied by a sudden decrease in blood pressure and can cause serious organ malfunction, may develop as a result. Sepsis is becoming more common, which suggests that it is a major global cause of severe disease and mortality [11]. According to a survey, people who recover from sepsis frequently suffer from permanent physical, mental, and cognitive impairments. Sepsis is a complex host response to a virus that is infecting a person that may be exacerbated by endogenous factors [12].

Concomitant AKI is a common complication of sepsis, with incidence rates ranging from 5 to 20 percent among hospital admissions and increasing to 35 to 50 percent among severely sick patients. In comparison to patients without septic AKI, these patients experience poorer outcomes, a greater risk of in-hospital death, and a higher level of long-term morbidity [13].

PenKid is a viable substitute for its physiologically active counterparts since it has a long half-life, is stable after collection, and is not affected by sex or age. PenKid is a potential biomarker for renal impairment in critically sick patients since it is not protein bound in plasma and is only filtrated in the glomerulus [14]. Thus, the aim of the current study was to assess penKid as a biomarker for sepsis related AKI in ICU patients.

Concerning the demographic characteristics (age and sex) as well as anthropometric measures (including height, weight, and BMI) in the current study, both groups demonstrated insignificant differences (P>0.05). Such outcomes indicated that both groups were comparable and both parameters (demographic characteristics and anthropometric measures) were not interfering with net results of the study.

Similar to this, **Hassan *et al.*** [15] assess plasma PenKid as a biomarker of septic AKI in patients admitted to the ICU. They proved that there was no discernible difference between the research groups (p=0.093).

In terms of the primary site of infection, UTI (44%) was demonstrated to be the most common cause followed by chest infection (40%) and then others.

Contrarily, according to research by **Rosenqvist and his collogues** [16], lower respiratory tract infections (33.5%) and urinary tract infections (21.9%) were the most common illnesses, followed by skin and soft-tissue infections (9.9%%) and upper respiratory tract infections (9.6 percent). In terms of Penkid levels between the two groups, the current investigation showed that septic AKI patients showed a substantial rise compared to the control group (P-value 0.001).

This was in line with original study done in 2021 by **Hassan and his colleagues** [15], who found that septic patients who had AKI had substantially higher plasma Proenkephalin levels on days 0, 2, and 7 after admission (P <0.001). They came to the conclusion that individuals with AKI had plasma PenKid levels that were significantly greater than those of patients without AKI. For the diagnosis of septic AKI, plasma PenKid is a useful early biomarker as a result. Additionally, Penkid levels in septic patients with chronic AKI were considerably higher (P <0.0001), according to research by **Hollinger and colleagues** [4]. Thus, they came to the conclusion that admission PenKid concentration was related to AKI in septic patients in a timely way.

The utility of AKI biomarkers in predicting AKI in a septic situation may thus be constrained by the way in which they interact with the inflammatory response. Penkid concentrations in patients without AKI are in the normal range, suggesting that the inflammatory response does not directly alter penkid. Therefore, compared to biomarkers impacted by the systemic inflammatory response, penkid may be more specific in septic AKI [17].

Penkid was further researched as a biomarker for the evaluation of kidney function and the prediction of the onset of AKI since it has been found that opioid receptors are strongly expressed in the kidney and that opioid agonists can boost kidney function. When compared to serum creatinine, penkid may be able to assess renal function more quickly and accurately [18].

Penkid has also demonstrated promising qualities in monitoring kidney function in critically unwell patients, especially those with sepsis. Penkid enhances the monitoring of kidney function in patients with acute heart failure in addition to reflecting the cardio-renal state following acute myocardial infarction and predicting AKI in the postoperative cardiac surgery situation [19].

The current study proved Penkid's efficacy for predicting the development of septic AKI by showing that it had a high accuracy AUC (AUC=0.985). The best cut off value was 1200.65 ng/ml, with accuracy of 93.8%, sensitivity of 90%, specificity of 100%, PPV of 90%, and NPV of 100%. In a similar vein, **Hassan *et al.*** [15] showed that the optimum plasma PenKid cutoff point for septic AKI was 145.0 pmol/l. With an area under the curve (AUC) of 0.796 (95% confidence range 0.667-0.924) (P-value 0.001), it had the greatest sensitivity (67.9%) and specificity (98.3%).

Also, the current study demonstrated that, AKI cases who required renal replacement therapy (RRT) showed higher level of penkid when compared to those who did not require (P <0.001), and, for the validity of Penkid for prediction of requirement of replacement therapy. Penkid showed high accuracy AUC (AUC=0.860), best cut off value of was 2609.4ng/ml. Agreement with our study, **Kim and his colleagues** [17] found that patients receiving RRT had considerably greater penkid concentrations than patients receiving no RRT (Penkid, 421.9 vs. 82.5 pmol/L, P-value 0.0001). Using ROC curve analysis, Penkid performed well in terms of RRT prediction (AUC, 0.872). In light of this,

they came to the conclusion that penkid is a highly accurate and objective biomarker of AKI and RRT and is helpful in predicting prognosis in patients with septic infections. Penkid is a potential biomarker in critical care conditions, including sepsis, because to its diagnostic resiliency and survival predictor power.

Similarly, **Caironi and his colleagues** [20] reported that, early measurement and the trajectory of penKid predict incident AKI, improvement of renal function, and the need for RRT in the acute phase after intensive care unit admission during sepsis or septic shock. PenKid measurement may be a valuable tool to test early therapies aimed at preventing the risk of AKI in sepsis.

In terms of the validity of Penkid for prediction of non-recovery outcome (CKD and/or death), creatinine, eGFR, CRP showed low accuracy AUCs (AUC=0.647, 0.628, and 0.660 respectively). Regarding penkid, high accuracy AUC was found (AUC=0.862), best cut off value of was 2609.4ng/ml, sensitivity was 84.2%, specificity was 90.3%, PPV was 84.2%, NPV was 90.3%, and accuracy was 88%. Thus, Penkid AUC was better than creatinine, eGFR and CRP for prediction of non-recovery septic AKI. In the same line, **Moledina and his colleagues** [3] demonstrated that, Penkid had a better AUC than serum creatinine in predicting non recovery outcome (0.78 vs. 0.64, P <0.001). Because patient harm could occur from failure to identify AKI using serum creatinine -based definition in sepsis, penkid could help risk stratify this subgroup missed by serum creatinine. For example, in those with admission serum creatinine ≤1.2 mg/dl, <5% of those with Penkid values <84.2 pmol/L experienced major adverse kidney event (MAKE), whereas about 20% of those with Penkid value >84.2 pmol/L experienced this outcome.

Additionally, **Hollinger and his colleagues** [4] revealed that, Penkid elevation preceded elevation of serum creatinine with worsening renal function (WRF) and was low in renal recovery. Thus they concluded that, admission penkid concentration was associated WRF in a timely manner in septic patients. With regards of overall survival (OS) Penkid has a significant negative correlation with the OS, being significantly decreased among recovery cases and significantly elevated among dead ones.

Marino *et al.* [21] reported that admission penkid concentration was increased in patients who died within seven days of admission, while procalcitonin and Cr clearance were not. In the present study, penkid concentration and eGFRs based on the four equations all showed significant differences between survivors and non-survivors.

The current study displayed that, Penkid level showed significantly positive correlation with baseline, after 48 h creatinine, CRP, SIRS and SOFA scores. Otherwise, no significant correlations were found between Penkid with other parameters in all studied cases.

Similarly, **Shah and his colleagues** [8] demonstrated that, penkid performs similarly to baseline creatinine in its ability to predict post-operative AKI. Importantly, penkid has a strong positive correlation with creatinine (r=0.806).

Due to delays in the discovery of decreased kidney function and the detection of AKI, early biomarkers are favoured over approaches dependent on an individual's urine output and blood creatinine to estimate glomerular filtration rate (GFR). In order to identify those who have septic AKI, the plasma penKid may be employed as an early biomarker [15].

CONCLUSION

PenKid was demonstrated to be a reliable surrogate promising biomarker for sepsis related AKI among unselected patients with sepsis. Additionally, penkid demonstrated superior advantage over creatinine, eGFR and CRP in terms of non-recovery septic AKI prediction.

LIMITATIONS

In spite of the promising analytical outcomes of the present study, the following limitations have to be considered: First, small sample size is the main drawback of the current study. Second, difficulties in appropriate diagnosis and selection of cases with sepsis according to the new definition. Third, inulin remains the gold standard for the assessment of kidney function but was not investigated in this study.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

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