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ORIGINAL ARTICLE

Urinary Liver Type Fatty Acid Binding Protein and Plasma Cystatin C Predict Acute Kidney Injury Outcomes in Critically Ill Patients

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ABSTRACT

Background: Acute kidney injury (AKI) is a common clinical syndrome affecting critically ill patients and associated with poor outcomes. AKI biomarkers have been developed with the aim of being able to detect kidney damage earlier than the detection process based on serum creatinine levels. Various AKI biomarkers have been discovered and validated to improve detection, differentiation, and progression of renal injury. Liver-type fatty acid-binding protein (L-FABP), which localizes in renal proximal tubules, is excreted into the urine in response to tubular injury. Cystatin C (Cys-C), a functional biomarker of AKI, is filtered through glomeruli and reabsorbed in the proximal tubule is independent of many factors such as age and muscle mass. This study aimed to assess the diagnostic and prognostic abilities of urinary L-FABP and plasma Cystatin C for detecting AKI and mortalities in critically ill patients. **Methods**: 44 patients aged 18 years or older admitted to the intensive care units (ICU) of the Department of internal medicine, Zagazig University Hospitals for more than 48 hours. Patients with a history of renal transplant, chronic dialysis, nephrectomy, acute kidney injury or chronic kidney disease at time of admission were excluded. All patients will be subjected to the following: Full clinical examination, pelvi-abdominal ultrasound and assessment of urinary L-FABP, plasma cystatin C and plasma creatinine(pCr), Acute Physiology and Chronic Health Evaluation II (APACHE II) Score and Sequential Organ Failure Assessment (SOFA) Score and the patients were observed for development of AKI or death within 30 days after ICU admission. **Results**:56.8% of patients developed AKI within 30 days after ICU admission. 27.3% of patients suffered from sustained AKI, 4.5% of patients required hemodialysis and 25% of patients died. Urinary L-FABP and plasma Cys-C were good predictive biomarker of AKI and death (P<0.001) and (P<0.001) respectively, while pCr was not predictive (P=0.393). **Conclusion**: Urinary L-FABP and plasma cystatin C have valuable diagnostic and prognostic information for detecting AKI and death in critically ill patients. Further multicenter studies on large numbers of patients are recommended.

Keywords: Liver Fatty Acid Binding Protein; Cystatin C; Critically Ill; Creatinine; Acute Kidney Injury

INTRODUCTION

Acute kidney injury (AKI) commonly affects critically ill patients and is strongly associated with increased risk of long-term loss of kidney function, mortality and morbidity [1]. Early diagnosis of AKI is important to establish appropriate therapies. Glomerular filtration rate (GFR) decreases progressively with the onset of kidney injury and is usually assessed by plasma creatinine and urine output. Creatinine is influenced by variety of renal and extrarenal factors, resulting in low diagnostic performance. Furthermore, the increased serum creatinine level is delayed which may limits therapeutic opportunities [2].

Different biomarkers were investigated for purpose of early detection of AKI. Some biomarkers were proved to be more effective compared to those currently used [3].

Liver-type fatty acid-binding protein (L-FABP), a 14kDa protein found in the cytoplasm of human renal proximal tubules, has been observed to bind free fatty acids in response to renal ischemia. Many studies demonstrated that L-FABP is a promising

biomarker of acute, and chronic kidney diseases [4].

Cystatin C (Cys-C); a protein with low molecular weight, is produced by all nucleated cells of the body, filtered by glomeruli, and is almost completely reabsorbed in the proximal tubule. Also, plasma Cys-C is independent of factors such as age, gender, and muscle mass. Cys-C is documented to be a good functional biomarker for detecting AKI [5].

This study aimed to evaluate the diagnostic and prognostic abilities of urinary L-FABP and plasma Cystatin C for detecting AKI, worse outcome, and mortalities in critically ill patients.

METHODS

This prospective observational study was conducted on 44 patients at the intensive care units of the Department of internal medicine, Zagazig University Hospitals (Egypt) from July 2019 to July 2020. Informed consent was obtained from all patients or their guardians. All procedures were performed in accordance with the directives of the Ethics Committee of Zagazig University Hospitals. Patients aged 18 years or older who were admitted to the ICU for more than 48 hours were considered for study inclusion whereas dialysis or renal transplant patients, acute or chronic kidney disease at time of admission were excluded from the study. All patients were subjected to full clinical examination and pelvi-abdominal ultrasound was performed to exclude chronic kidney disease. The following investigations were done; urinary L-FABP, plasma Cystatin C, plasma creatinine (pCr), complete blood count, liver function test and arterial blood gases. Urine samples for L-FABP and blood samples for Cys-C were collected on entry to the ICU and after 24 and 48 hours and centrifuged at 1000 g for 15 min, before being stored at − 20 °C until assayed and measured using enzyme-linked immunosorbent assay (ELISA) kits, as per manufacturer protocol.

AKI was defined as per the kidney disease: Improving Global Outcomes (**KDIGO**) guidelines for AKI [6]. Assessment of severity of illness using Acute Physiology and Chronic Health Evaluation II (APACHE II) Score [7] and Sequential Organ Failure Assessment (SOFA) Score [8]. Data about renal impairment and mortality were collected for 30 days.

STATISTICAL ANALYSIS

Data were analyzed using computerized software statistical packages (SPSS version 20). Mean was used for quantitative data. Qualitative data was described by numbers & percentages. Chi-Square (X^2) used to compare proportions. The independent sample t-test to compare means. Multivariate analysis and linear correlation were performed. Analysis of Variance (ANOVA) and post-hoc used. (ROC) curve; receiver operating characteristics evaluated cut-off; cystatin and L-FABP values as predictors of AKI, sensitivity, specificity, positive and negative predictive value (PPV), (NPV). P-value less than 0.05 was considered highly significant.

RESULTS

After application of exclusion criteria, the total number of the study participants was 44 patients (23 patients (52.3%) were females while 21 patients (47.7%) were males. The age of patients ranged from 24 to 85 years old, and the mean age was 56 years. 56.8% of patients developed AKI after admission to the ICU. 12 patients (27.3%) suffered from sustained AKI, two patients (4.5%) required hemodialysis (HD) and eleven patients (25%) died (**Table 1**). However, no differences were noted between patients with and without AKI with respect to gender, AKI patients were statistically significantly older than patients without AKI (**Table 2**).

Area under the receiver-operating curve (AUC-ROC) values for uL-FABP were 0.856 (95% confidence interval (CI): 0.717 to 0.943) with a sensitivity of 96% (95% CI, 79.6 - 99.9%) and a specificity of 3.16% (95% CI, 38.4 - 83.7%), P= <0.001 with positive predictive value (PPV) of 77.4 (95% CI, 65.4 to 86.1) and negative predictive value (NPV) of 92.3 (95% CI, 63.0 to 98.8). The AUC of pCys-C was 0.823 (95% CI: 0.679 to 0.922) with a sensitivity of 84% (95% CI,63.9 - 95.5%) and a specificity of 89.47% (95% CI,66.9 - 98.7%), P= <0.001 with PPV of 91.3 (95% CI, 73.7 to 97.5) and NPV of 81.0 (95% CI, 63.1 to 91.4) (**Table 3**, **Figure S1**).

There was a statistically significant increase in the mean value of L-uFAPB and Cys-C concentration after 24h, also after 48h, in contrast to pCr, there was no statistically significant increase in the mean value of pCr concentration after 24h and the increase started to be statistically significant after 48h (**Table 4**).

uL-FABP and pCys-C were predictive of sustained AKI (P<0.001). Also, the mean value of basal uL-FABP and pCys-C were predictive of death within 30 days than in survivors (P<0.02 and P=0.036 respectively) (**Table 5**).

The mean value of uL-FABP concentrations was statistically significant with pCys-C and APATCHE II as regard composite outcome and the mean value of pCys-C concentrations was statistically significant with uL-FABP only **(Table 6)** respectively while pCr concentrations was not associated with age (**Table S1**).

Risk prediction was assessed using univariable and multivariable logistic regression for the clinical model. The clinical model is shown in (**Table 7**) and contains a priori selected predictors including age, sex, and serum creatinine at the time of biomarker Measurement, and APACHE II score. After adjustment, uL-FABP acted as an independent predictor of composite outcome.

Table 1: Characteristics and outcome of the studied population (N= 44)

		Total number= 44	
		N	%
Age, years		56 (24-85)	
Sex	Female	23	52.3%
	Male	21	47.7%
Diagnosis	Cerebrovascular	11	25.0%
	Cardiopulmonary	11	25.0%
	Metabolic	8	18.2%
	Sepsis	10	22.7%
	Malignancy	4	9.1%
Composite Outcome of the studied population. Death and/or AKI (N= 44)	Totally affected	25	56.8%
	Sustained AKI	12	27.3%
	Hemodialysis	2	4.5%
	Death	11	25%

AKI: acute kidney injury

Table 2: Comparison of clinico-demographic data as regard the Composite Outcome

		Composite Outcome Death and/or AKI				Total N=44		Test	P
		Yes N=25		No N=19					
		N	%	N	%	N	%		
Age		57 (36-85)		48 (24-69)		56 (24-85)		-2.04	0.041
Sex	Female	14	56.0 %	9	47.4 %	23	52.3%	0.32	0.570
	Male	11	44.0 %	10	52.6 %	21	47.7%		
Diagnosis	Cerebrovascular	6	24.0 %	5	26.3 %	11	25.0%	4.5	0.337
	Cardiopulmonary	5	20.0 %	6	31.6 %	11	25.0%		
	Metabolic	3	12.0 %	5	26.3 %	8	18.2%		
	Sepsis	8	32.0 %	2	10.5 %	10	22.7%		
	Malignancy	3	12%	1	5%	4	9%		

Table 3: The validity of pCys-C and pCr levels with area under the ROC curve (AUC) as a diagnostic marker for Composite Outcome

	Cutoff	Sensitivity% 95% CI	Specificity % *95% CI*	PPV 95% CI	NPV 95% CI	AUC 95% CI	P
pCys-C mg/L	>2.1	84 63.9 - 95.5	89.47 66.9 - 98.7	91.3 73.7 - 97.5	81.0 63.1 - 91.4	0.823 0.679 -0.922	<0.001
uL-FABP ng/L	17.3	96 79.6 - 99.9	63.16 38.4 - 83.7	77.4 65.4 - 86.1	92.3 63.0 - 98.8	0.856 0.717 to 0.943	<0.001
pCr mg/dl	>0.71	56 34.9 - 75.6	73.68 48.8 - 90.9	--	--	0.636 0.47 - 0.77	0.105

pCys-C plasma cystatin-C. **pCr**: plasma creatinine. **uL-FABP**: urinary liver-type fatty acid-binding protein. **PPV: positive** predictive value. **NPV**: negative predictive value.**CI** confidence interval. **AUC** area under curve

Table 4: Comparison of uL-FABP, pCys-C & pCr levels at baseline and on follow up in patients with and without Composite Outcome

patients with Composite Outcome					
Markers	0-Hr	24-Hr	48-Hr	F	P
uL-FABP ng/L	85.6 (15.6-160.7)	95.4 (18.9-189.1)*	109.2 (18.8-205.8) * *	17.1	<0.001
pCys-C mg/L	6.30 (0.51-46.70)	6.50 (0.74-52.30) *	7.40 (0.60-50.00) * *	5.4	<0.001
pCr mg/dl	0.72 (0.42-1.10)	0.77 (0.40-1.26)	0.93 (0.23-1.53) * *	17.7	0.009
patients without Composite Outcome (no AKI OR Death)					
uL-FABP	14.4 (2.9-93.3)	15.2 (6.9-113.1)	23.1 (4.4-118.5)	1.4	0.246
pCys-C mg/L	1.10 (0.49-10.80)	1.00 (0.50-10.10)	0.91 (0.50-7.90)	0.05	0.949
pCr mg/dl	0.91 (0.38-1.22)	0.69 (0.23-1.2)	0.83 (0.40-1.3)	0.271	0.674

pCys-C plasma cystatin-C. **pCr**: plasma creatinine. **uL-FABP**: urinary liver-type fatty acid-binding protein. **LSD** Post-hoc test: *** * P** statistically significant when 48-hour levels compared with either 24-hours or 0-hours levels. *** P** statistically significant when 24-hour levels compared with 0-hours levels.

Table 5: Comparison of composite outcome as regard the Baseline L-FABP and Baseline CysC Levels based on ROC curve

		Baseline CysC Level				Total N=44		X2 Test	P
		Low N=20		High N=24					
		N	%	N	%	N	%		
Mortality	Died	2	10%	9	37.5%	11	25 %	4.4	0.036
	Alive	18	90 %	15	62.5%	33	75%		

		Baseline CysC Level				Total N=44		X2 Test	P
		Low N=20		High N=24					
		N	%	N	%	N	%		
AKI	Yes	2	10%	19	79.2%	21	47.7%	20.9	<0.001
	No	18	90%	5	20.8%	23	52.3%		
		Baseline L-FABP Level				Total N=44			
		Low N=12		High N=32					
		N	%	N	%	N	%		
Mortality	Died	0	0.0%	11	34.4%	11	25.0%	4.9	0.02
	Alive	12	100%	21	65.6%	33	75.0%		
AKI	Yes	0	0.0%	21	65.6%	21	47.7%	15.1	<0.001
	No	12	100.0%	11	34.4%	23	52.3%		

pCys-C: plasma cystatin-C. **pCr:** plasma creatinine. **uL-FABP:** urinary liver-type fatty acid-binding protein

Table 6: Correlations between baseline **uL-FABP** and **pCys-C** levels with certain studied parameters within each group

	Baseline uL-FABP				Baseline pCys-C			
	Composite Outcome				Composite Outcome			
	Yes		No		yes		no	
	r	P	r	P	r	P	r	P
pCys-C mg/L	0.486	0.014	0.336	0.16	--	--	--	--
uL-FABP ng/L	--	--	--	--	0.486	0.014	0.336	0.16
pCr mg/dl	0.242	0.243	0.008	0.974	0.329	0.108	-0.238	0.327
Age	0.043	0.838	0.116	0.636	-0.050	0.813	-0.206	0.397
APATCHE II	0.553	0.014	0.172	0.41	-0.126	0.547	0.502	0.029
SOFA	0.254	0.22	0.27	0.248	0.308	0.134	0.124	0.612

pCys-C plasma cystatin-C. **pCr**: plasma creatinine. **uL-FABP**: urinary liver-type fatty acid-binding protein. **APATCHE**: Acute Physiology And Chronic Health Evaluation. **SOFA**: Sequential Organ Failure Assessment

Table 7: Univariate and multivariate logistic regression of potential predictors of Composite Outcome

	Univariate analysis			Multivariate analysis		
	RR	95% C.I. for RR	P	RR	95% C.I. for RR	P
Sex (M vs F)	0.71	0.21-2.34	0.571			
Age	0.95	0.90-1.00	0.032	1.90	1.82-3.00	0.040
uL-FABP* ng/L	0.96	0.93-0.98	0.002	1.96	1.93-3.00	0.048
pCys-C* mg/L	0.72	0.56-0.92	0.009	0.80	0.59-1.10	0.171
pCr mg/dl	0.06	0.00-2.72	0.148			

	Univariate analysis			Multivariate analysis		
	RR	95% C.I. for RR	P	RR	95% C.I. for RR	P
APATCHE II	0.86	0.78-0.95	0.003	1.08	0.88-1.33	0.461
SOFA	0.53	0.35-0.80	0.003	0.64	0.33-1.23	0.179

***** Used as a continuous variable. **pCys-C** plasma cystatin-C. **pCr**: plasma creatinine. **uL-FABP**: urinary liver-type fatty acid-binding protein. **APATCHE**: Acute Physiology And Chronic Health Evaluation. **SOFA**: Sequential Organ Failure Assessment. **RR** relative risk

DISCUSSION

AKI is a devastating problem that is frequently associated with poor outcomes in critically ill patients. Depending on the traditional markers for diagnosis of AKI, an undesirable delay in the diagnosis and treatment has occurred. So, it is challenging to find a biomarker for early diagnosis of AKI [9].

Twenty-five ICU patients (56.8%) included in our study were identified as acute kidney injury patients. The prevalence of AKI is increasing in recent years, nearly 3-20% for general inpatients and 30-60% critically ill patients [10].

Our study observed that patients with AKI were older than non-AKI patients. The results of our study are comparable to those reported by Yokota et al [11]. No difference was noted between patients with and without AKI with respect to gender. This finding agrees with that reported by Grams et al [12].

As in this study, SOFA and APACHE-II scores were found to be associated with 30- day morbidity and mortality in patients with AKI. APACHE-II and SOFA scores are one of the most used predictive scoring systems for critically ill patients and have been widely used in predicting their prognosis [13].

Liver-type fatty acid binding protein is a protein that plays a role in the free fatty acid metabolism. It is found in the proximal tubules and increases during AKI [14].

The use of urinary L-FABP as a diagnostic biomarker in patients with AKI, is increasing. In patients treated with repair of abdominal aortic aneurysm **Obata et al**. showed that urinary LFABP was a sensitive marker of AKI, and basal urinary L-FABP can predict the development of postoperative AKI [15]. Similarly, **Hishikari et al**, suggested that the baseline urinary L-FABP level may predict AKI in acute heart failure patients [16].

In patients with rapid deterioration of kidney function, higher levels of urinary L-FABP were associated with need of renal replacement therapy or worse outcomes. Also, clinical studies found that L-FABP level can be used as a predictor of contrast-associated AKI [17]**.**

We also found that higher L-FABP levels were independently associated with 30-day mortality after ICU admission. Doi et al. demonstrated that urinary biomarkers including LFABP were able to predict 14-day mortality in critically ill patients [18].

In this study, plasma Cys-C served as a functional biomarker with high specificity for AKI detection and prognosis prediction. Several studies showed that the overall diagnostic sensitivity and specificity was 0.82 (95% CI: 0.75 to 0.87) and 0.82 (95% CI: 0.78 to 0.86), respectively. The overall area under the receiver operating characteristic curve reached 0.89 [19].

In most studies Serum Cys-C was found to be a better marker for early diagnosis of AKI compared to creatinine. Analysis of data of 982 patients who developed AKI in prospective cohort studies, from 15 countries, plasma cystatin C was highly predictive for all-cause AKI [20]. Also, the increase of cystatin C more than 10 %, predicted serious adverse outcome; death or requiring dialysis even without an associated increase in plasma creatinine [21].

Cystatin C has a shorter half-life compared to creatinine, so it acquires a steady-state equilibrium more rapidly. These characteristics allowed plasma cystatin C to be an alternative or in combination with plasma creatinine as a marker of renal function [20].

CONCLUSION AND RECOMMENDATION

In summary, this study investigated the diagnostic and predictive role of urinary LFABP and plasma Cys-C for renal function and their potential application in the clinical settings. Urinary L-FABP and plasma cystatin C provided valuable diagnostic and prognostic information among critically ill patients. Conducing more multicenter

studies on large numbers of patients is recommended.

Conflict of Interest: None.

Financial Disclosure: None.

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SUPPLEMENTARY TABLES AND FIGURES

Table S1: Correlations between baseline pCr levels and certain studied parameters within each group

	Baseline pCr			
	Composite Outcome			
	Yes		No	
	r	P	r	P
uL-FABP ng/L	0.242	0.243	0.008	0.974
pCys-C mg/L	0.329	0.108	-0.238	0.327
Age	0.762	<0.001	-0.096	0.649
APATCHE II	-0.218	0.295	0.190	0.435
SOFA	0.089	0.671	0.310	0.196

pCys-C plasma cystatin-C. **pCr**: plasma creatinine. **uL-FABP**: urinary liver-type fatty acid-binding protein. **APATCHE**: Acute Physiology And Chronic Health Evaluation. **SOFA**: Sequential Organ Failure Assessment. **RR** relative risk

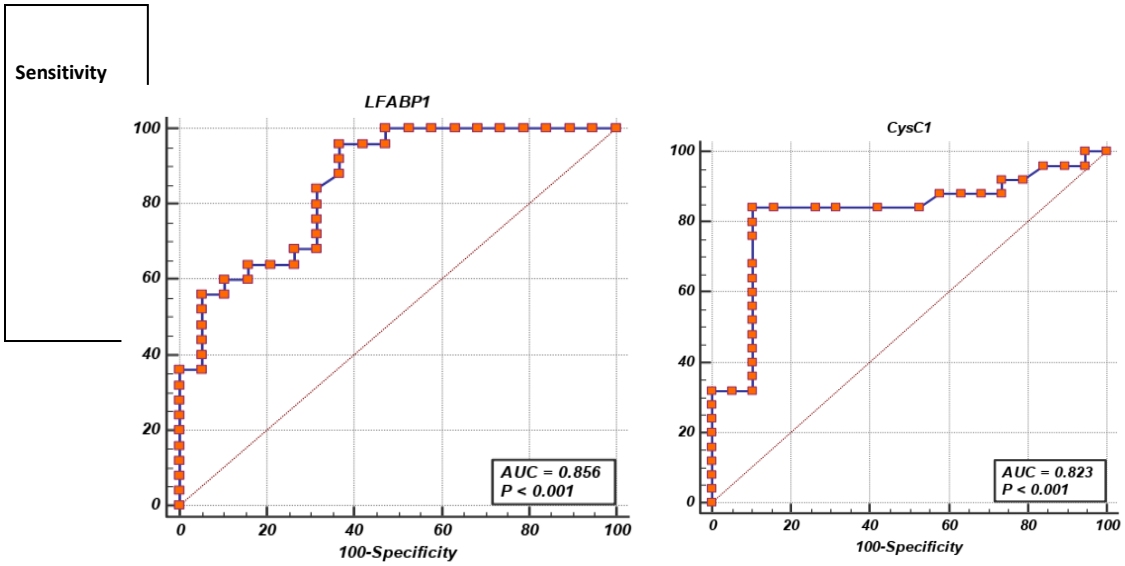

Figure S1: ROC curve of **uL-FABP** and **pCys-C** at baseline as markers for composite outcome in critically ill patients. **pCys-C** plasma cystatin-C**. uL-FABP**: urinary liver-type fatty acid-binding protein