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Urinary interleukin-18 as a biomarker of Acute Kidney Injury in the Critically Ill Patients

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ABSTRACT

Urinary IL-18 is a biomarker that is elevated in patients with acute tubular necrosis and does not seem to be affected by prerenal azotemia or chronic kidney disease. The present study was designed to evaluate the diagnostic utility of urinary IL-18 as an early marker of AKI in critically ill patients. A total of 120 critically ill patients were included in this observational prospective randomized study. All patients were subjected to a measurement of Urinary IL-18 and the sCr at fashioned time intervals to assess which can predict early renal impairment. There was a significant difference in urinary IL-18 level between patients with AKI and patients who did not develop AKI on admission and 6 and 24 hours from admission ($p < 0.001$ for all). Moreover, there was a significant increase of urinary IL-18 level after 6 and 24 hours in both group ($p < 0.001$ for all). There was no significant difference in sCr level on admission and after 6 hours between both groups ($p=0.817$ and 0.760 respectively), while after 24 and 48 hours, sCr level was significantly higher in AKI group ($p < 0.001$ for both). Serum Cr level start to increase significantly after 24 hours in AKI group ($p < 0.001$). The most sensitive in detecting AKI was urinary IL-18 after 6 and 24 hours of the admission with a sensitivity of 100% and a specificity of 100%. So urinary IL-18 can be used as a test for the early diagnosis of AKI in critically ill patients.

Key words: Urinary IL-18, acute kidney injury, APACHE II

INTRODUCTION

Acute kidney injury (AKI) is a common complication in critically ill patients, affecting 25% of intensive care unit (ICU) admissions, and is associated with high mortality rates of around 40–50% (De Mendonca A *et al.,* 2000). It is interesting to note that the mortality from renal failure has not changed markedly over the years despite the development and introduction of new techniques. There are several reasons for this including the lack of effectiveness of new therapies and late diagnosis (Ympa Y *et al.,* 2005, Vinsonneau C *et al.,* 2006).

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Serum creatinine (sCr) is the most widely used parameter for everyday assessment of glomerular filtration rate (GFR), but it has poor sensitivity and specificity in AKI because sCr lags behind both renal injury and renal recovery (Lameire N *et al.,* 2004). It comes into play as a marker for decreasing kidney function only after more than 50% of kidney function has been lost and is only useful after a steady state has been reached, which can take time especially in ICU patients (Honore P *et al.,* 2007). In addition changes in sCr can be nonspecific as they may occur as a result of several non-renal factors, such as muscle mass and nutrition (Chertow R *et al.,* 2003). Though, sCr is an unreliable and delayed marker of the deterioration of kidney function during AKI as it does not directly reflect cell injury, but rather the delayed functional consequences of the damage (Chirage R *et al.,* 2006).

It is important to prevent and to early manage even the mildest forms of AKI to preserve renal functions, to prevent complications of AKI and to prevent the need for chronic dialysis (Venkataraman R *et al.,* 2005). An early detection of patients with kidney injury may provide the opportunity to treat and prevent the extension of kidney injury well before the sCr rises**.** Thus, there is a need for rapidly available, sensitive, and specific biomarkers for AKI that would allow early prediction at a time when

intensive care optimization can be performed (Devarajan P *et al.,* 2009, Sampurna M *et al.,* 2009).

Recent studies have proposed many biomarkers for early detection of AKI**.** Such novel biomarkers may facilitate earlier diagnosis and proper management resulting in fewer complications and improved outcomes (Soni S *et al.,* 2009).

Interleukin-18 (IL-18) is a cytokine that produced by macrophages and other cells. It is a pro-inflammatory cytokine that encoded by the IL-18 gene and works by binding to the IL-18 receptors, and induces cell-mediated immunity.

IL-18 was cloned from a murine liver cell cDNA library generated from animals primed with heat-killed Propioni-bacterium acnes and subsequently challenged with lipopolysaccharide (Okamura H *et al.,* 1995). Nucleotide sequencing of murine IL-18 predicted a precursor polypeptide of 192 amino acids lacking a conventional signal peptide and a mature protein of 157 amino acids. Subsequent cloning of human IL-18 cDNA revealed 65% homology with murine IL-18 and showed that both contain an unusual leader sequence consisting of 35 amino acids at their N terminus (Ushio S *et al.,* 1996).

IL-18 possesses broad and potent immunomodulatory properties. It appears essential to host defenses against a variety of infections. IL-18 is particularly effective during the clearance of intracellular bacteria, fungi and protozoa requiring the induction of host-derived IFN-γ, which in turn evokes effector pathways involving molecules such as nitric oxide. IL-18 also plays a part in the clearance of viruses, partly through the induction of cytotoxic T cells with viral clearance being impaired in IL-18 deficient mice (Nakamura K *et al.,* 1993).

Urinary IL-18 was increased in mice with ischemic AKI (Fantuzzi G *et al.,* 2013). Also, it has been shown to be elevated in patients with acute tubular necrosis and does not seem to be affected by prerenal azotemia, urinary tract infection, or chronic kidney disease (Zhou H *et al.,* 2006).

The aim of the current study is to evaluate the diagnostic utility of urinary IL-18 as an early marker of AKI in critically ill patients and to determine its ability to predict clinical AKI compared with traditional sCr.

PATIENTS AND METHODS

Patients:

One hundred and twenty critically ill patients admitted to the Critical Care department, Cairo University hospital, Cairo, Egypt were enrolled in this observational prospective randomized study during the period from December 2014 to June 2016. The study was approved by the institution review board at Cairo University. Informed consent was obtained from all patients or their close relatives.

Exclusion criteria included patients < 18 years, those with chronic kidney disease or history of renal transplantation, use of nephrotoxic drugs, or those received RRT at any time before admission, those with end stage liver disease or terminal malignancy, those on corticosteroid therapy, and post cardiopulmonary resuscitation patients.

Patients who did not meet the exclusion criteria were included into this study, and they were followed up for the development of AKI during their stay in ICU according to the Acute Kidney Injury Network (AKIN) criteria (Bagshaw

S *et al.,* 2008 and Gulati A *and Bagga A.,* 2008) (table-1).

The AKIN group, an international collaboration of nephrologists and intensivists, have proposed the term AKI to represent the spectrum of acute renal dysfunction ranging from mild elevation in sCr to severe forms requiring renal replacement therapy (RRT). The network also attempted to develop consensus on diagnostic criteria and staging of AKI (Nakamura K *et al.,* 1993, Gulati A and Bagga A*.,* 2008).

It is necessary that the diagnosis be made following estimation of at least two creatinine values within 48 hour (Gulati A and Bagga A*.,* 2008). Individuals who receive RRT are considered to have met the criteria for stage 3 irrespective of the stage they are in at the time of RRT and only one criterion (creatinine or urine output) should be fulfilled to qualify for a stage (Bagga A *et al.,* 2007). The baseline sCr should be the lowest creatinine value recorded within 3 months of the event (If the baseline sCr value is not available within 3 months and AKI is suspected so, repeat sCr within 24 hours) (Lewington A *et al.,* 2011).

Evaluation of Patients:

All included patients were subjected to the following:

1. Full Clinical Evaluation: Including history and physical examination.
2. Abdominal ultrasonography: to exclude chronic kidney & liver disease.
3. Laboratory investigations:
   - Routine Labs: *CBC (complete blood count):* Hemoglobin, Hematocrit, White blood cells, and platelet count; *Coagulation profile:* PT, PC and INR; *Kidney Function Tests:* Na, K, sCr, and blood Urea; *Liver Function Tests*: ALT (Alanine aminotransferase), AST (Aspartate aminotransferase) and total and direct bilirubin; and arterial blood gas (ABG). All routine labs measured on admission except sCr which measured on admission, 6, 24 and 48 hours after admission.
   - Labs specific for this study:

- Estimated creatinine clearance using Cockcroft-Gault formula: Creatinine clearance (mL/min) = [(140 – age) x weight/72 x (sCr)] x 0.85 if female. (Rigalleau V *et al.,* 2005)
- Urinary IL-18 was measured for all patients quantitavely by ELISA technique on admission, 6 and 24 hours after admission and expressed as pg/ml. (Devarajan P *et al.,* 2009).

Sample collection and storage:

The sample of urine was aseptically collected into a sterile container. Centrifuged to remove particulate matter, assayed immediately or aliquot and stored at -20˚C.

Reagent preparation:

Wash Buffer: If crystals was formed in the concentrate, it was warmed to room temperature and mixed gently until the crystals have completely dissolved. 30 mL of wash buffer concentrate was diluted into deionized or distilled water to prepare 750 mL of wash buffer.

Standard: The Standard was reconstituted with 1.0 mL of Sample Diluent. This reconstitution produced a stock solution of 1000 pg/mL. The standard was allowed to settle for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard served as the high standard (1000 pg/mL). The Sample Diluent served as the zero standards (0 pg/mL).

Test procedure:

Reagent A and B were diluted to the working concentration using assay Diluent A and B (1:100), respectively. All reagents were allowed to reach room temperature. Appropriate numbers of strips for experiment was removed from micro-titer plate. Removed strips were resealed and stored at 4˚C. 100 µl of Sample was added per well, covered with the Plate sealer and incubated for 2 hours at 37˚C. The liquid of each well was removed. 100 µl of detection reagent A working solution was added to each well, covered with the Plate sealer, incubated for 1 hour at 37˚C, warmed to room temperature and mixed gently until solution appeared uniform. Each well was aspirated and washed. The process was repeated three times for a total of three washes. Each well was washed by filling with wash buffer (approximately 400µl). After the last wash, any remaining wash buffer was removed by aspiration or decanting. 100 µl of detection reagent B working solution was added to each well, covered with a new plate sealer and incubated for 1 hour at 37˚C. 90 µl of substrate solution was added to each well, covered with a new plate sealer, incubated within 15-30 minutes at 37˚C and protected from light. 50 µl of stop solution was added to each well. The optical density of each well was determined spectro-photo-metrically at a wave length of 450 nm ± 2 nm. The concentration of IL-18 in the samples was then determined by comparing the optical density of the samples to the standard curve.

Application of scoring System:

APACHE II score (Beck D *et al.,* 1997), which is a severity of disease classification system, was evaluated on the day of admission. After admission, an integer score is computed based on several measurements; higher scores imply a more severe disease and a higher risk of death.

The statistics:

Continuous variables were summarized using range and mean ± SD. Categorical variables were summarized using frequencies and relative frequencies. Continuous variables were compared using the two-sample t-tests (for two variables) or ANOVA test (for more than two variables), and categorical variables were compared using the Chi-square and Fisher exact test. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. Correlation between various variables was done using Person correlation equation for non-normal variables. A probability value (*p* value) of less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 for Microsoft Windows.

RESULTS

Demographic and baseline clinical data at ICU admission: (Table-2)

Urinary IL-18 and baseline clinical characteristics:

There was no significant correlation between urinary IL-18 and age (r = 0.155, p = 0.092). Moreover, there was no significant differences seen in the urinary IL-18 and sex (p = 0.842), hypertension (p = 0.578), diabetes millets (p = 0.892) or comorbidities (p = 942). (Table-4) On the other hand, a significant correlation was found between Urinary IL-18 level and APACHE II score (r = 0.401, p < 0.001).

Urinary IL-18 level and sCr in AKI and non AKI subjects:

There was a significant difference in urinary IL-18 level between patients with AKI and patients who did not develop AKI on admission and 6 and 24 hours from admission.

Table-1. Classification/staging system of AKI by AKIN criteria

Stage	Creatinine criteria	Urine output
1	Rise of serum creatinine by > 0.3 mg/dl (>26.4 umol/l) or increase to ≥ 150-200% (1.5-fold to twofold) from baseline	<0.5 ml/kg per hour for > 6h
2	Increase of serum creatinine by > 200-300% (twofold to threefold) from baseline	<0.5 ml/kg per hour for > 12h
3	Increase of serum creatinine to > 300% (> threefold) from baseline (or serum creatinine ≥ 4 mg/dl (≥ 354 umol/l with an acute rise of at least 0.5 mg/dl (44 umol/l)	<0.3 ml/kg per hour for 24h, or anuria for 12 h

Table-2. Demographic and clinical data of patients entered into the study:-

Characteristics		Value
Age: (mean±SD {range} in years)		48.3 ± 16.2 {19-70}
Male: Female sex (Nº of patients)		66: 54
Vital signs (mean±SD)		
HR		105 ± 17
MBP		93 ± 20
Temperature		37.7 ± 0.4
RR		20 ± 5
Hypertension (Nº of patients (%))		38 (31.6 %)
DM (Nº of patients (%))		18 (15 %)
Comorbidities (Nº of patients (%))		
Cardiovascular causes		38 (31.6 %)
Cerebrovascular strokes		35 (29.1 %)
Sepsis		30 (25 %)
Trauma		16 (13.3 %)
Metabolic		28 (23.3 %)
Scoring systems		
GCS **(**mean±SD (range))		11.63 ± 3.33 (3-15)
APACHE II score **(**mean±SD (range))		9.19 ± 6.11 (0-33)
AKIN Stage **(**Nº of patients (%))		
Baseline	Stage I	12 (13.4 %)
	Stage II	78 (86.6 %)
After 24 hours	Stage I	6 (6.7 %)
	Stage II	67 (74.4 %)
	Stage III	17 (18.9 %)
Laboratory Data (mean±SD)		
Platelets		250808 ± 61076
Leukocytic count		10945 ± 3815
Hematocrit		37.85 ± 5.01
Hemoglobin		11.94 ± 1.78
INR		1.11 ± 0.17
PH		7.32 ± 0.09
PaO2		85.17 ± 10.01
AST		35.9 ± 9.69
ALT		31.9 ± 8.83
Bilirubin (Direct)		0.14 ± 0.04
Bilirubin (Total)		0.84 ± 0.12
Potassium		3.74 ± 0.42
Sodium		140.1 ± 4.2
Urea		49.7 ± 21.6
GFR		102.3 ±16.7
Creatinine		0.89 ± 0.14
IL-18		49.51 ±3.86
Outcome (Nº of patients (%))		
	AKI	90 (75 %)
	Non AKI	30 (25 %)

N: number; **ST:** standard deviation; **HR:** Heart rate; **MAP:** Mean arterial pressure; **RR:** Respiratory rate; **DM:** diabetes mellitus; **GLS:** Glasgow coma scale; **APACHE II *score***, Acute Physiology And Chronic Health Evaluation; **AKIN,** Acute Kidney Injury Network; **ALT:** alanine aminotransferase, **AST:** aspartate aminotransferase; **GFR,** glomerular filtration rate; **IL-18,** Interleukin-18**; PaO_2,** partial pressure of arterial oxygen; **AKI**, acute kidney injury.

Moreover, there was a significant difference between admission urinary IL-18 level and its level after 6 and 24 hours in both group ($p < 0.001$ for all) (Table 3).

On the other hand, there was no significant difference in sCr level on admission and after 6 hours between both groups (p=0.817 and 0.760 respectively), while after 24 hours and 48 hours, sCr level was significantly higher in AKI group ($p < 0.001$ for both). (Table-3).

There was no significant difference between admission sCr level and its level after 6 hours in AKI group ($p = 0.123$). There was a significant difference between admission sCr level and its level after 24 and 48 hours in AKI group. ($p < 0.001$ in both). On the other hand, there was no significant difference between admission sCr level and its level after 6, 24 and 48 hours in non AKI group. (Table-3).

There was significant correlation between urinary IL-18 and sCr levels 24 hours from admission ($r = 0.924$ with $p < 0.001$). On the other hand, there was no significant correlation between admission urinary IL-18 and admission sCr levels and their levels 6 hours from admission ($r = 0.138$ and 0.144 with $p = 0.134$ and 0.117 respectively).

There was significant correlation between urinary IL-18 and sCr levels 24 hours from admission in the AKI group ($r = 0.270$ with $p = 0.01$). On the other hand, there was no significant correlation between admission urinary IL-18 and admission sCr levels and their levels 6 hours from admission in AKI group ($r = 0.029$ and 0.168 with $p = 0.786$ and 0.079 respectively).

There was no significant correlation between admission urinary IL-18 and admission sCr levels and their levels 6 and 24 hours from admission in non AKI group ($r = 0.058$, 0.263 and 0.372 with $p = 0.762$, 0.161 and 0.051 respectively).

Prognostic ability of urinary IL-18:

The receiver operator characteristic (ROC) curve was calculated for the use of admission and 6 and 24 hours urinary IL-18 levels as a predictor of AKI. The area under the ROC (AUROC) curve for admission urinary IL-18 to predict AKI was 0.997 (95% confidence interval, 0.986 – 1.0) with an optimal cutoff value 47.4 pg/ml. This cutoff value gave a sensitivity of 96% and a specificity of 100% for ICU. (Fig-1).

Table-3. Demographic and clinical data of patients entered into the study:-

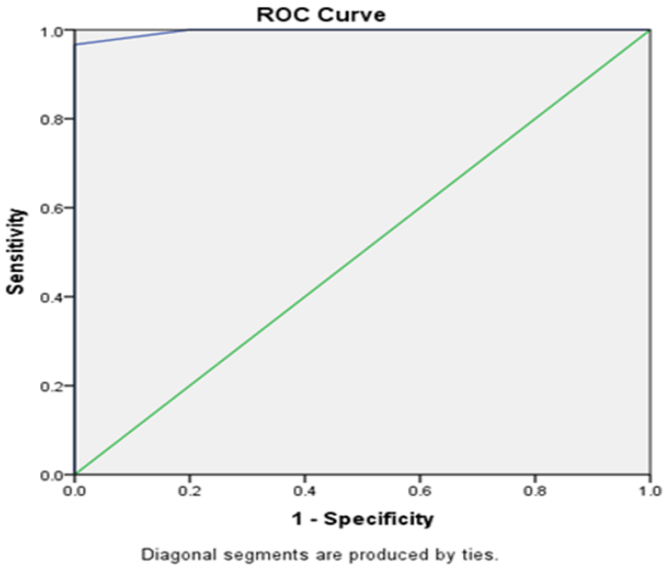
Characteristics	NO AKI (N. 30)	AKI (N. 90)	P value
Age: (mean±SD {range} in years)	48.2 ± 13.6 {25-65}	45.1 ± 17.7 {19-70}	0.481
Male: Female sex (Nº of patients)	17: 13	49:41	0.574
Vital signs (mean±SD)			
HR	93.00 ± 8	109 ± 17	<0.001
MBP	99 ± 12	88 ± 24	0.027
Temperature	37.3 ± 0.3	37.8 ± 0.7	0.001
RR	15 ± 2	22 ± 6	<0.001
Hypertension (Nº of patients (%))	11 (36.6 %)	27 (30 %)	0.496
DM (Nº of patients (%))	6 (20 %)	12 (13.3 %)	0.375
Scoring systems			
GCS **(**mean±SD (range))	13 ± 1.8 (10-15)	10 ± 3.9 (3-15)	0.001
APACHE II score **(**mean±SD (range))	4.5 ± 2.75 (0-8)	12.36 ± 7.04 (4-33)	<0.001
Laboratory Data (mean±SD)			
Platelets	235800±24478	245766±65039.34	0.430
Leugocytic count	9423.33 ± 1554.01	11036.66 ± 4368.49	0.075
Hematocrit	39.30 ± 4.27	37.73 ± 5.21	0.051
Hemoglobin	12.40 ± 1.68	11.73 ± 1.89	0.167
INR	1.14 ± 0.25	1.10 ± 0.14	0.530
PH	7.38 ± 0.07	7.31 ± 0.10	0.002
PaO2	90.80 ± 2.87	82.00 ± 11.78	<0.001
AST	35.03 ± 4.66	37.50 ± 11.48	0.189
ALT	30.73 ± 3.69	33.31 ± 9.69	0.241
Bilirubin (Direct)	0.14 ± 0.05	0.13 ± 0.03	0.143
Bilirubin (Total)	0.80 ± 0.67	0.84 ± 0.13	0.158
Potassium	3.79 ± 0.21	3.73 ± 0.47	0.474
Sodium	140.56 ± 4.21	139.56 ± 4.28	0.333
Urea	44.10 ± 16.27	51.13 ± 26.22	0.148
GFR	110.93 ±14.41	102.57 ±19.24	0.048
Creatinine			
On admission	0.89 ± 0.14	0.88 ± 0.14	0.817
After 6 hours	0.91 ± 0.11	0.93 ± 0.18	0.760
After 24 hours	0.93 ± 0.13	1.95 ± 0.18	<0.001
After 48 hours	0.94 ± 0.10	2.22 ± 0.58	<0.001
	* 0.554	* 0.123	
	** 0.250	** 0.000	
	*** 0.135	*** 0.000	
IL-18			
On admission	44.43 ±1.30	51.09 ±2.83	<0.001
After 6 hours	54.20 ±3.76	99.03 ±12.14	<0.001
After 24 hours	66.01 ±4.44	134.50 ±17.32	<0.001
	$^{+,++,+++}$ <0.001	$^{+,++,+++}$ <0.001	

N: number; **ST:** standard deviation; **HR:** Heart rate; **MAP:** Mean arterial pressure; **RR:** Respiratory rate; **DM:** diabetes mellitus; **GLS:** Glasgow coma scale; **APACHE II *score***, Acute Physiology And Chronic Health Evaluation; **ALT:** alanine aminotransferase, **AST:** aspartate aminotransferase; **GFR,** glomerular filtration rate; **IL-18,** Urinary Interleukin-18**;** **PaO_2,** partial pressure of arterial oxygen; **AKI**, acute kidney injury. ***** Significance between admission serum creatinine and serum creatinine after 6 hours; ****** Significance between admission serum creatinine and serum creatinine after 24 hours; ******* Significance between admission serum creatinine and serum creatinine after 48 hours; $^{+}$ Significance between admission IL-18 and IL-18 after 6 hours; $^{++}$ Significance between admission IL-18 and IL-18 after 24 hours; $^{+++}$ Significance between IL-18 after 6 hours and IL-18 after 24 hours.

Table-4. Comparison between admission urinary IL-18 and Demographic and clinical data of patients entered into the study.

Characteristics	Urinary IL-18 (mean±SD)	P value
Gender		
Male	49.5±4.1	0.842
Female	49.2±3.5	
Comorbidities		
Cardiovascular causes	48.8±3.4	
Cerebrovascular strokes	49.3±5.9	
Sepsis	49.1±4.5	0.942
Trauma	47.3±2.6	
Metabolic	48.8±4.6	
Hypertension		
Yes	49.4±4.3	0.578
No	48.8±3.8	
Diabetes mellitus		
Yes	49.1±4.9	0.892
No	49.2±3.3	

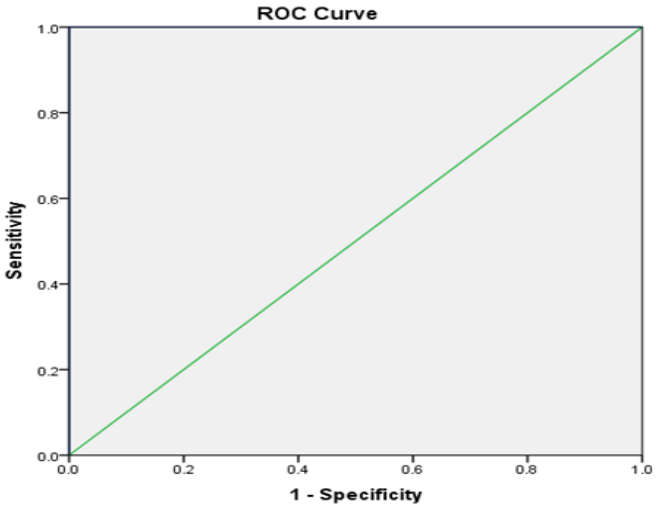
Figure-1. ROC curve of urinary IL-18 on admission to predict AKI



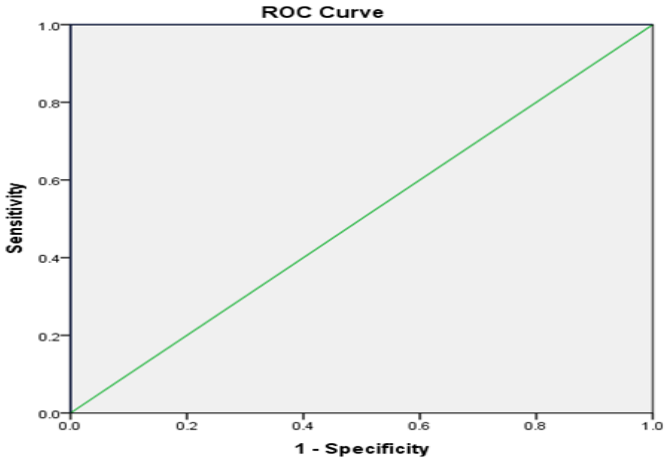
The area under the ROC (AUROC) curve for urinary IL-18 6 hours after admission to predict AKI was 1 (95% confidence interval, 1.0 – 1.0) with an optimal cutoff value 70 pg/ml. This cut off value gave a sensitivity of 100% and a specificity of 100% for ICU (Fig-2).

The area under the ROC (AUROC) curve for urinary IL-18 24 hours after admission to predict AKI was 1 (95% confidence interval, 1.0 – 1.0) with an optimal cutoff value 85.5 pg/ml. This cutoff value gave a sensitivity of 100% and a specificity of 100% for ICU. (Fig-3).

Figure-2. ROC curve of urinary IL-18 at 6 hours after admission to predict AKI



Figuree-3. ROC curve of urinary IL-18 at 24 hours after admission to predict AKI



DISCUSSION

IL-18 levels in the kidneys increase more than doubles following AKI and the conversion of IL-18 precursor to the mature form requires caspase-1 (Melnikov V *et al.,* 2001). IL-18 blocking antibodies decreased injury to a similar degree as seen in caspase-deficient mice. These results demonstrate that IL-18 is an important mediator of acute ischemic AKI. The source of IL-18 in kidney injury is thought to be the proximal tubule (Edelstein CL *et al.,* 2007) and not macrophages (He Z *et al.,* 2009), neutrophils or CD4 T cells (Melnikov V *et al.,* 2001 and Faubel S *et al.,* 2005).

Previous studies show that IL-18 is released into the urine at the time of renal tubular cell injury, and can be measured through noninvasive methods. (Hall I *et al.,* 2010 and Zhou H *et al.,* 2006). The availability of such biomarker increased our interest to conduct the current study to assess the diagnostic utility of urinary IL-18 as an early predictor of AKI in critically ill patients, in order to allow early intervention to treat AKI.

Current study included patients with age ranged from 19-70 years with mean age (48.3±16.2). There was no significant correlation between IL-18 and age and gender.

This is in agreement with Chirag R et al., (2005) who found that urinary IL-18 level was not influenced by age in a study included 332 patients with mean age 51±18.6, P = 0.9. Also, the result is in agreement with Hongqi R et al., (2015) who found that urinary IL-18 level was not related to gender in a study included 95 patients, 75 males (79%) and 20 females (21%) (P = 0.481).

Patients included in the study admitted to ICU with different comorbidities and there was no correlation between urinary IL-18 levels and comorbidities (P value 0.942).

This is in contrast with Xin L et al., (2015) who made a meta-analysis and found that, there were significant correlation between cardiac surgery patients who developed AKI in ICU and high levels of urinary IL-18 (p = 0.008).

There was a significant correlation between admission urinary IL-18 level and APACHE II score (P < 0.001). This goes hand by hand with Hongqi R et al., (2015) who found a significant correlation between IL-18 and APACHE II score (P value < 0.001).

Concerning urinary IL-18 as a biomarker for AKI in ICU patients, we compared its level in AKI and non AKI patients on admission, 6 and 24 hours after admission and have found that IL-18 levels significantly increased in all times in patients who developed AKI as defined by AKIN criteria (P < 0.001 for all). Moreover, there was a statistically significant increase in the urinary IL-18 level from 51.09±2.83 on admission to 99.03±12.14 after 6 hours and 134.50±17.32 after 24 hours in patients with AKI (P < 0.001 for all).

This observation was in agreement with a prospective multicenter cohort study made by Chirag R et al, (2011) which involved 1291 adult patients undergoing cardiac surgery. Urine and blood samples were collected preoperatively and daily for up to 5 postoperative days. For the first 24 hours postoperatively urine samples were collected every 6 hours. There were 60 patients that developed AKI as documented by receiving acute dialysis or doubling of sCr. It was found that the first postoperative sCr was equal to or lower than its baseline level in 60% of the cohort, while urinary IL-18 level was higher in AKI patients at each time point and peaked in the first collections (0-6 hours). It was found that a postoperative level of urinary IL-18 >60 pg/ml denoted > 6 fold risk of AKI, where ROC curve was calculated and AUC was 0.76 [95% CI(1.9-24.3)] with a p = 0.03.

Pathophysiological mechanisms contributing to AKI after cardiac surgery include diminished renal blood flow, loss of pulsatile flow, hypothermia, athero-embolism and a generalized inflammatory response. Various clinical algorithms have been proposed for prediction of AKI, based on preoperative risk factors, but objective tests for the early diagnosis of lesser degrees of renal injury are not widely available (Lassnigg A *et al.,* 2004; Thakar C *et al.,* 2005 and Ranjith KT et al, 2017).

Also, the above observation go with a study that was held in the cardiology department of Alexandria university hospital by Mohamed S et al., (2012) that included 112 patients who underwent elective cardiac catheterization from which 20 patients developed AKI. Urine samples were collected before, 2 hours and 4 hours after the procedure and urinary IL-18 was measured by ELISA. It was found that there was no significant difference between IL-18 levels when measured before catheterization and 2 hours after catheterization but significant difference was found when it was measured 4 hours after catheterization, P < 0.001.

Also agreed with a study by Jeffrey C et al., (2013) that was conducted on 40 perioperative liver transplant patients for early detection of AKI with other markers including IL-18. Samples were taken 24 hours pre-operatively and 24 hours post-operatively and IL-18 was measured in urine samples by ELISA. IL-18 level in pre-operative samples were statistically similar when compared between AKI and non-AKI groups (P = 0.427), but in post–operative samples IL-18 levels were significantly high in AKI group with a median level of 883.09 pg/ml (AUC 0.749, P = 0.044).

Also goes with a study made by Ling W et al., (2008) which conducted on 150 patients that had coronary angiography using low-osmolar contrast medium. Urine samples were collected before and 24 hours after coronary angiography and IL-18 levels measured by ELISA. Contrast induced nephropathy was diagnosed in 13 patients and urinary IL-18 level was significantly increased in this group but not in the control group (P<0.05). The predictable time of AKI onset determined by IL-18 was 24 hours earlier than determined by sCr. (P<0.05).

On the other hand, this study was in contrast with a study by Siew E et al., (2010) that was conducted on 451 ICU patients from which 86 patients developed AKI within 48 hours of enrollment. The AUC for urinary IL-18 predicting subsequent AKI within 24 hours was 0.62 [95%CI (0.54-0.69)]. The highest median urinary IL-18 levels were observed in patients with sepsis at enrollment, those received acute dialysis and those who died within 28 days of ascertainment. So it was found that urinary IL-18 did not reliably predict AKI development but did predict poor clinical outcomes in a broadly selected critically ill adult patients.

Also, the result of the current study do not agree with a prospective cohort study made by Michael H et al., (2008) that was conducted on 100 adult cardiac surgical patients undergoing cardiopulmonary bypass at a tertiary hospital. SCr and urinary IL-18 levels were measured preoperatively, on arrival to ICU and 24 hours postoperatively. It was found that 20 patients developed AKI on arrival to the ICU and 24 hours postoperatively urinary IL-18 was not different in patients who subsequently developed AKI compared with those who did not. On arrival to ICU AUC was 0.53 [95%CI (0.38 - 0.68)], P = 0.70. At 24 hours postoperatively AUC was 0.55 [95%CI (0.40- 0.71)], P =0.48. denoting that IL-18 was not a good predictor of AKI, instead it was

significantly correlated with duration of cardiopulmonary bypass (P <0.001).

ROC curve was done to validate the usage of urinary IL-18 as a predictor of AKI on admission, 6 hours and 24 hours after admissions. The AUCs were 0.997, 1 and 1 respectively denoting a good prediction with cut-off points of 47.4 pg/ml, 70 pg/ml and 85.5 pg/ml respectively.

This is in agreement with Mohammad S et al., (2012) who found that AUC was 1 with a cut-off point of 55.66 pg/ml 4 hours after admission. Also, Chirag R et al., (2011) found that AUC was 0.76 with a cut-off point of 60 pg/ml within 6 hours of admission and Jeffery C et al., (2013) found that AUC was 0.749 with a cut-off point of 883.09 pg/ml 24 hours after admission.

Limitation of the study:

A relatively low number of subjects. The limitations of slow and insensitive change of sCr to detect renal injury are well known, and therefore future studies will have to correlate urinary IL-18 not only with sCr but also with established markers of renal injury and function such as urine microalbuminuria, fractional excretion of sodium, or measured GFR.

CONCLUSIONS

Urinary IL-18 may be considered as a reliable test for early diagnosis of AKI in critically ill patients nearly 24 hours before any significant rise in sCr and can be consider as a helpful tool for early diagnosis, prevention and treatment of AKI.

ABBREVIATIONS:

AKI, acute kidney injury; ICU, intensive care unit; **sCr,** serum creatinine; **GFR,** glomerular filtration rate; **IL-18,** Interleukin-18; **AKIN,** Acute Kidney Injury Network; **RRT,** renal replacement therapy; **APACHE II *score***, Acute Physiology And Chronic Health Evaluation; **ROC**, receiver operator characteristic; **ELISA**, enzyme linked immune sorbent assay.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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