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RESEARCH ARTICLE

URINARY INTERLEUKIN-18 AS A BIOMARKER FOR EARLY DIAGNOSIS OF ACUTE KIDNEY INJURY IN INTENSIVE CARE UNIT IN ZAGAZIG UNIVERSITY HOSPITALS

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Abstract

BACKGROUND:Acute Kidney Injury (AKI) can occur spontaneously or iatrogenically, and rates of AKI continue to rise over the last two decades despite improvements in clinical care and development of preventive strategies. Diagnosis of AKI by serum creatinine is likely too late to prevent some of the early structural changes that characterize renal injury. 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. Currently, the detection of a reliable biomarker for early diagnosis of AKI would assist in facilitating early intervention, evaluating the effectiveness of the therapeutic intervention, and guiding pharmaceutical development. Urinary IL-18 was increased in mice with ischemic AKI compared to sham-operated mice. Thus we developed the hypothesis that IL-18 could be released from the injured tubular epithelial cells into the urine and serve as a urinary biomarker of AKI in humans. **OBJECTIVE:** The aim of this study is to Estimate U IL-18 in critically ill patients who will be admitted in I.C.U, to Assess validity of U IL-18 in early prediction of AKI and Compare the U IL-18 with s.creatinine (at admission, 6h and 24h after admission). **METHODS**: A total number of 84 patients that were critically ill who were admitted to medical ICU of Zagazig University Hospitals within 6 months and were classified into 2 groups :1) **AKI group** : The s.creatinine level was elevated either by 25% or more of the basal level or by ≥0.3mg/dl above the basal level after 24 hours and It included 36 patients (22 males and 14 females) with age ranged from 29 years to 60 years with a mean values ± SD of 47.7±8.8years.

2) **Non AKI group** : Rise of the s.creatinine level less than 0.3mg/dl above the basal level after 24 h. It included 48 patients (31 males and 17 females) with age ranged from 22 years to 53 years with mean values ± SD of 42.6±10.6 years. S.creatinine and U IL-18 were measured basal at admission , 6 hours and 24 hours after. **RESULTS**: U IL-18 was significantly increased in AKI group after 6 hours compared to its basal values. Further increase of U IL-18 after 24 hours compared to both basal and 6 hours values. We also revealed that UIL-18 was higher in patients with AKI compared to patients without AKI. S.creatinine was not elevated in either groups after 6 hours. S.creatinine was significantly increased in AKI after 24 hours. We found that the ***sensitivity*** and ***specificity*** of U IL-18 at 6h from admission in ICU was 91.1% and 93.9% respectively. **CONCLUSIONS**: Urinary IL-18 (pg/dl) is significantly increased in AKI groups , this increase is earlier than

any rise of serum creatinine in these patients . Urinary IL-18 is more specific and sensitive in prediction of AKI than serum creatinine , So urinary IL-18 can be used as an early biomarker of AKI in critically ill patients in ICU that may allow early prediction at a time when intensive care optimization can be performed and that can help in prevention of worsening of these cases and good outcome.

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Introduction

Acute kidney injury (AKI) is common and the absolute incidence of AKI has increased during the last decade. Between 5% and 40% of critically ill patients in the intensive care unit (ICU) have an episode of AKI. Up to 4.9% of critically ill patients in the ICU will require renal replacement therapy. AKI requiring renal replacement therapy in the ICU has a high mortality of over 50%. The commonest causes of AKI are septic shock, ischemia and nephrotoxins **(1).** Acute kidney injury (AKI) has been defined conceptually as a rapid decline in glomerular filtration rate (GFR) that occurs over hours or days **(2)**. In the RIFLE criteria for AKI, AKI consists of three graded levels of injury (Risk, Injury and Failure) based upon either the magnitude of elevation in serum creatinine or urine output and two outcome measures (Loss and End-stage renal disease). The RIFLE strata are as follows:

Risk: 1.5-fold increase in the serum creatinine or GFR decrease by 25 percent or urine output <0.5 mL/kg per hour for six hours.

Injury: Two fold increase in the serum creatinine or GFR decrease by 50 percent or urine output <0.5 mL/kg per hour for 12 hours.

Failure: Three fold increase in the serum creatinine or GFR decrease by 75 percent or urine output of <0.5 mL/kg per hour for 24 hours, or anuria for 12 hours.

Loss: Complete loss of kidney function (e.g. need for renal replacement therapy) for more than four weeks.

ESRD: Complete loss of kidney function (e.g. need for renal replacement therapy) for more than three months **(3).**

Hospital-acquired acute kidney injury (AKI) is a common complication that is associated with significant morbidity and mortality **(4)**. Hospital stay is longer in patients with AKI and patients are more likely to be discharged to short or long term care facilities **(5,6).** A wealth of epidemiological data has accumulated that AKI is independently associated with increased mortality. Increased in-hospital mortality occurs in patients with both mild **(7)** and severe (requiring renal replacement therapy) AKI **(8,9).**

Although AKI is clearly associated with increased risk of in-hospital and long term mortality, the mechanisms by which AKI contributes to death are unclear. It is possible that the development of AKI is a marker of susceptibility to injury or of the severity of underlying illness. However, emerging clinical and experimental data are accumulating that AKI contributes to distant organ injury. Thus, the high mortality of AKI may be due to deleterious short and long term systemic effects **(10).**

Interleukin-18 (IL18) is a cytokine that produced by macrophages and other cells. It is a proinflammatory cytokine that encoded by the IL-18 gene and works by binding to the interleukin 18, and induces cell-mediated immunity **(11).** IL-18 has been implicated as an inflammatory mediator of hashimoto thyroiditis, the most common cause of autoimmune hypothyroidism. IL-18 has also been found to increase the Alzheimer's disease-associated amyloid-beta production in human neuron cells**.** IL-18 levels are very stable in several pathological conditions: no significant changes were seen in urine of patients with neoplastic diseases, central nervous system disorders, infections, rheumatoid arthritis and other disorders **(12).**

Immunohistochemistry of mouse kidneys demonstrated an increase in IL-18 protein in injured tubular epithelial cells in AKI kidneys compared to normal controls. In a separate study using freshly isolated proximal tubules from mice, it was determined that hypoxic proximal tubules had high levels of IL-18**(13).**

On the basis of the demonstration of IL-18 in injured proximal tubules, IL-18 was measured in the urine. Urine IL-18 was increased in mice with ischemic AKI compared to sham-operated mice. Thus we developed the hypothesis that IL-18 could be released from the injured tubular epithelial cells into the urine and serve as a urinary biomarker of AKI in humans**(14).**

Therefore, the aim of this study to evaluate urinary IL-18 as an early sensitive biomarker of acute kidney injury in critically ill-patients in ICU where their previous serum creatinine was normal.

PATIENTS AND METHODS:

This work had been carried out in medical intensive care unit of Internal Medicine department, Faculty of Medicine, Zagazig University hospitals.

Study design:

Observational descriptive cohort and analytic study where critically ill patients admitted to the medical ICU of Zagazig university hospitals in the period between May 2013 to November 2013 were included and assessed in the study. Participants were enrolled after written consent.

Patients:

A total number of 84 patients admitted to medical ICU of Zagazig University Hospitals within 6 months .

The patients were classified into 2 groups :

- **AKI group** : The creatinine level was elevated either by 25% or more of the basal level or by ≥0.3mg/dl above the basal level after 24 hours.

It included 36 patients (22 males and 14 females) with age ranged from 29 years to 60 years with a mean values ± SD of 47.7±8.8years. Their BMI ranged from 23 to 27 with a mean value±SD of 25.3±2.1.

- **Non AKI group** : Rise of the serum creatinine level less than 0.3mg/dl above the basal level after 24 h.

It included 48 patients (31 males and 17 females) with age ranged from 22 years to 53 years with mean values ± SD of 42.6±10.6 years. Their BMI ranged from 24 to 27 with mean value±SD of 25.5±1.4.

Exclusion critieria :

- All subjects were selected to be free from hepatic diseases, renal diseases , rheumatic diseases , malignancies , hypertension, thyroid diseases and diabetes mellitus.

Methods:

All subjects of the study were subjected to the following:-

A) Full medical history taking and thorough clinical examination: According to checking patient's records.

B) Routine investigations:

They were all done according to the methods applied in the clinical pathology laboratories of Zagazig university hospitals and included:

1-Complete Urine analysis.
2-Complete blood picture.
3-Random plasma glucose level and HbA1C (American Diabetes Association ,2011).
4-Liver function tests.
5- Renal function tests**:** serum creatinine basal on admission, 6 hours and 24 hours after admission, serum urea by colorimetric method.
6-Calcuation of glomerular filtration rate using MDRD equation:
eGFR (mL/min/1.73 m2) = **175** x $(Scr)^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female), basal at admission, 6 hours and 24 hours after.
7- TSH.
8- Pelvi-abdominal ultrasound.

C) Specific investigations*:* included

Measurement of urinary IL-18 by ELISA basal at admission , 6 hours and 24 hours after.

1.Test principle:

- This Enzyme-Linked Immunosorbent Assay (ELISA) allows the quantitative determination of human Interleukin-18 from urine, plasma and serum.
- The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-18. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-18 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain IL-18, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color.

The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of IL-18 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.Specimen collection , preparation and storage of reagents:-

-Sample collection and storage :-

Aseptically collect the sample of urine, voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at -20˚C with avoidance repeated freeze-thraw cycles.

-**Reagent preparation :-**

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (1000 pg/mL). The **Sample Diluent** serves as the zero standard (0 pg/mL).

3. Test procedure:

Reagent A and B - Dilute to the working concentration using **Assay Diluent A and B** (1:100), respectively.

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37˚C directly). **All reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and store at 4˚C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

1. Add 100 µl of **Standard**, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37˚C.
2. Remove the liquid of each well, don't wash. Add 100 µl of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37˚C. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37˚C.
5. Repeat the aspiration/wash as in step 4.
6. Add 90 µl of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within 15-30 minutes at 37˚C. Protect from light.
7. Add 50 µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

4. Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph.

The data may be liberalized by plotting the log of the IL-18 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 1.3. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Statistical analysis:

Data were analyzed with SPSS version 15.0 (statistical package for the Social Science, Chicago, IL). Quantitative data were expressed as mean ± standard deviation (SD) or standard error (SE). SE=SD/square root of patients number which was used in case of big SD, data were analyzed by independent sample, paired t test and one way analysis of variance (ANOVA). While qualitative data were expressed as number and percentage and were analyzed by Chi square (X2) test. Correlation was done using Pearson correlation test. The receiver operating characteristic (ROC) curve and 95% confidence interval (CI) was performed to determine cutoff values for the studied biomarkers. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. P-value was considered significant if <0.05 and highly sign.

RESULTS:

The clinical and laboratory data of studied groups (AKI & Non AKI) after admission in ICU are presented in **Table (1)**.The results showed that No statistically significant differences were found between studied groups (AKI &Non AKI) as regard sex, Age **,** BMI, Hb, ALT and AST ,basal serum creatinine (mg/dl) and basal urinary IL-18 (pg/dl) .

Table (2) : shows comparison between the basal and 6h after admission in ICU values of the studied biomarkers in Non AKI and AKI groups. where, there was no statistically significant difference in the urinary IL-18 (pg/dl)

(t=1,79,p>0.05) and serum creatinine (mg/dl) (t=0.01,p>0.05) in patients without AKI and there was a statistically high significant difference in the urinary IL-18 (pg/dl) (t=8.6, p<0.001) , while there was no a statistically significant difference in serum creatinine (mg/dl) (t=0, p>0.05) in patients with AKI.

Table (3): Shows comparison between the basal and 24h after admission in ICU values of the studied biomarkers in Non AKI and AKI groups, where, There was no statistically significant difference in the urinary IL-18 (pg/dl) (t=1.7, p>0.05) and serum creatinine (mg/dl) (t=0.01, p>0.05) in patient without AKI. while, There was a statistically high significant difference in the urinary IL-18 (pg/dl) (t=15.1, p<0.001) and serum creatinine (mg/dl) (t=12.5, p<0.001) in patients with AKI.

Table (4): Comparison between the 6h and 24h from admission in ICU as regarding Urinary IL-18 and serum creatinine values in Non AKI and AKI group.

There was a statistically high significant difference in the urinary IL-18 (pg/dl) between the studied groups (AKI & Non AKI) at 6h and 24h after admission in ICU and there was a statistically significant difference in the serum creatinine (mg/dl) between the studied groups (AKI & Non AKI) at 24h after admission in ICU.

Table (5): Correlation of Urinary IL-18 (pg/dl) after 6h versus some studied parameters in Non AKI and AKI group, shows no statistically significant correlations between 6h urinary IL-18 (pg/dl) versus each of Age (Years) (r=0.22 p>0.05), basal serum creatinine (mg/dl) (r=0.15, p>0.05) , Hb level (g/dl) (r=-0.149, p>0.05), basal urinary IL-18 (pg/dl) (r= 0.27, p>0.05) and MDRD (ml/min) (r=-0.23, p>0.05) in Non AKI group and also shows no statistically significant correlations between 6h urinary IL-18 (mg/l) versus each of Age (Years) (r=0.33, p>0.05) and Hb level (g/dl) (r=-0.149, p>0.05) whereas statistically high significant correlations were found versus basal serum creatinine (mg/dl) (r=0.69, p<0.001) and basal urinary IL-18 (pg/dl) (r=0.65, p<0.001). whereas a statistically significant correlation was found versus MDRD (ml/min) (r=-0.54, p<0.05) in AKI group.

Table(6) : Correlation of Urinary IL-18 (pg/dl) after 24h versus some studied parameters in Non AKI and AKI group. shows no statistically significant correlations between 24h urinary IL-18 (pg/dl) versus each of Age (Years) (r=0.23, p>0.05) , basal serum creatinine (mg/dl) (r=0.17, p>0.05), Hb level (g/dl) (r=-0.165, p>0.05) , basal urinary IL-18 (pg/dl) (r=0.33, p>0.05) , and MDRD (ml/min) (r=-0.24, p>0.05) in Non AKI group. While, in AKI patients no statistically significant correlations between 24h urinary IL-18 (pg/dl) versus each of Age (Years) (r=0.35, p>0.05) and Hb level (g/dl) (r=-153, p>0.05) whereas statistically high significant correlations were found versus basal serum creatinine (mg/dl) (r=0.71, p<0.001) and basal urinary IL-18 (pg/dl) (r=0.68, p<0.001) and statistically significant correlation was found versus MDRD (ml/min) (r=-0.52, p<0.05).

Table (7) : shows the time course of the studied markers among patients without and with AKI by using ANOVA test. Where, there were no statistically significant difference along the time course for urinary IL-18 (pg/dl) (F=0.01, p>0.05) and creatinine (mg/dl) (F=0 , p>0.05) in patient without AKI. while, there was a highly statistically significant difference along the time course for urinary IL-18 (pg/dl) (F=58, p<0.001) and serum creatinine (mg/dl) (F=69.75, p<0.001) in patient with AKI

Table (8) : shows the validity of the urinary IL-18 (pg/dl) as predictor of AKI after 6 hours. Setting a cutoff value of 65.5 (pg/dl) for urinary IL-18 (pg/dl) yielded a sensitivity and specificity of 91.1% and 93.9% respectively with PPV of 75% and NPV of 94.1%.

Fig (1) : shows the Area under the ROC curve of urinary IL-18 (pg/dl)=0.94 (95%CI; 0.86 – 1.02) at 6 hours from admission in ICU.

Table (1) : Demographic and Laboratory data of studied groups after admission in ICU using independent t- test.

		AKI group (n=36) (Mean±SD)	No AKI (n=48) (Mean±SD)	t-test	P
Sex	Male	22(64%)	31(66%)		

	Female	14(42%)	17 (34%)	3	NS*
Age (Years)		47.7±8.8	42.6±10.6	1.8	NS*
BMI (Kg/m^2) (18.5 – 24.9)		25.3±2.1	25.5±1.4	0.47	NS*
ALT (IU/L) (10- 40)		28.3±6.2	31.8±6.8	0.85	NS*
AST (IU/L) (10 – 40)		25.5±7.08	27.6±6.8	0.59	NS*
Hb (g/dl) (12-17)		13±1.3	13.1±1.1	0.99	NS*
Basal serum creatinine (mg/dl)		0.84±0.06	0.81±0.05	1.57	NS*
Basal urinary IL-18 (pg/dl)		52.29±4.49	53.87±11.21	1.66	NS*

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table (2) : Comparison between the basal and 6h From admission in ICU as regarding Urinary IL-18 and serum Creatinine values in Non AKI and AKI groups.

Biomarker	**Non AKI**				**AKI**			
	Basal	6hs	Paired-t test	P	Basal	6hs	Paired-t test	P
Urinary IL-18 (pg/dl)	53.87±11.21	58.06±11.25	1.97	0.45 NS	52.29±4.49	95.4±20.34	8.6	<0.001 **HS**
Serum Creatinine (mg/dl)	0.81±0.05	0.82±0.07	0.01	0.9 NS	0.84±0.06	0.84±0.07	0	0.9 NS

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table (3) : Comparison between the basal and 24h From admission in ICU as regarding Urinary IL-18 and serum Creatinine values in Non AKI and AKI group.

Biomarker	Non AKI				AKI			
	Basal	24hs	Paired-t test	P	Basal	24hs	Paired-t test	P
Urinary IL-18 (pg/dl)	53.87±11.21	60.13±11.24	1.7	0.11 NS	52.29±4.49	127.05±27.9	15.1	<0.001 **HS**
Serum Creatinine (mg/dl)	0.81±0.05	0.82±0.06	0.01	0.9 NS	0.84±0.06	1.9±0.09	12.5	<0.001 **HS**

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table(4) : Comparison between the 6h and 24h from admission in ICU as regarding Urinary IL-18 and serum creatinine values in Non AKI and AKI group.

	AKI group	Non AKI group	T- test	P
6hrs				
Urinary IL-18 (pg/dl)	95.41±20.34	58.06±11.25	23	**HS** <0.001
Serum Creatinine (mg/dl)	0.84±0.06	0.82±0.07	1	NS >0.05
24hrs				
Urinary IL-18 (pg/dl)	127.05±27.9	60.13±11.24	30	**HS** <0.001
Serum Creatinine (mg/dl)	1.9±0.09	0.82±0.06	12.5	**S** < 0.05

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table(5): Correlation of UrinaryIL-18(pg/dl) after 6 hours versus some studied parameters in Non AKI and AKI group.

6hrs urinary IL-18(pg/dl) Versus	Non AKI		AKI	
	r	p	r	P
Age (yrs)	0.22	>0.05 NS	0.33	>0.05 NS
Basal serum creatinine (mg/dl)	0.15	>0.05 NS	0.69	<0.001 **HS**

Basal urinary IL-18(pg/dl)	0.27	>0.05 NS	0.65	<0.001**HS**
Hb (g/dl)	-0.149	>0.05 NS	-0.149	>0.05 NS
MDRD (ml/min)	-0.23	>0.05 NS	-0.54	<0.05 **S**

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table(6): Correlation of Urinary IL-18 (pg/dl) after 24 hours versus some studied parameters in Non AKI and AKI groups.

24hrs urinary IL-18(pg/dl) Versus	**Non AKI**		**AKI**	
	R	p	r	P
Age (yrs)	0.23	>0.05 NS	0.35	>0.05 NS
Basal serum creatinine (mg/dl)	0.17	>0.05 NS	0.71	<0.001 **HS**
Basal urinary IL-18(pg/dl)	0.33	>0.05 NS	0.68	<0.001 **HS**
Hb (g/dl)	-0.165	>0.05 NS	-0.153	>0.05 NS
MDRD (ml/min)	-0.24	>0.05 NS	-0.52	<0.05 **S**

S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table (7) : Time-course of the studied markers among patients without AKI and with AKI by using ANOVA test.

Patients category	Biomarker	Basal	6hs	24hs	F	P
Patients without AKI	urinary IL-18 (pg/dl)	53.87±11.21	58.06±11.25	60.13±11.24	0.01	0.9 NS
	Serum Creatinine (mg/dl)	0.81±0.05	0.82±0.07	0.82±0.06	0	1 NS
Patients with AKI	urinary IL-18 (pg/dl)	52.29±4.49	95.41±20.34	127.05±27.9	58	<0.001 **HS**
	Serum Creatinine (mg/dl)	0.84±0.06	0.84±0.07	0.85±0.09	69.75	<0.001 **HS**

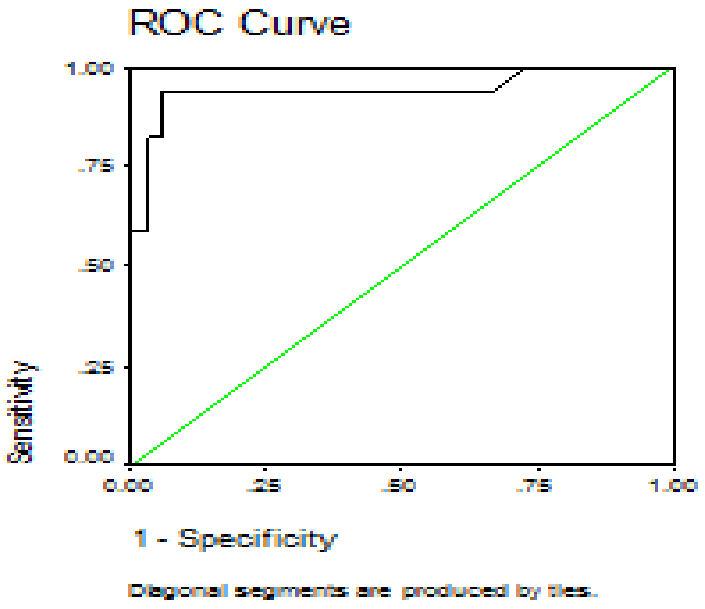
S: Significant (p value < 0.05) **NS***: Non significant (p value > 0.05) **HS**: Highly significant (p value < 0.001)

Table (8) : Validity of U IL-18 (6h) as predictor of acute kidney injury.

			AKI		Total
			+ve	-ve	
U IL-18	ve+	≥69.5 (pg/dl)	31	5	36
	ve-	<69.5 (pg/dl)	5	43	48
Total			36	48	84

*sensitivity →91.1% *specificity →93.9
*PPV→75 *NPV→94.
*Kappa→0.715 *P=0.000

Figure (1) :AUC of urinary IL-18 =0.94 (95%CI; 0.86 – 1.02) as a biomarker to detect AKI



DISCUSSION:

Acute Kidney Injury (AKI) , previously referred to as acute renal failure (ARF) , represents a significant and devastating problem in clinical medicine. The incidence of AKI varies from (5%) of hospitalized patients to 30–50% of patients in intensive care units **(15).**

Often AKI manifests as a transient rise in serum creatinine and is managed conservatively; however, a group of patients, often with significant co morbidity, require temporary renal replacement therapy (**16).** Acute renal injury is typically diagnosed by measuring serum creatinine. Unfortunately, creatinine is an unreliable indicator during acute changes in kidney function **(17).**

The proposed diagnostic criteria for AKI are an abrupt (within 48 hours) absolute increase in the serum creatinine concentration of ≥ 0.3 mg/dL from baseline, a percentage increase in the serum creatinine concentration of ≥ 25 percent, or oliguria of less than 0.5 mL/kg per hour for more than six hours **(2)**.

The latter two of these criteria are identical to the RIFLE "risk" criteria. The addition of an absolute change in serum creatinine of ≥ 0.3 mg/dL is based on epidemiologic data that have demonstrated an 80 percent increase in mortality risk associated with changes in serum creatinine concentration of as little as 0.3 to 0.5 mg/dL **(4)**.

However, animal studies have shown that whereas acute kidney injury can be prevented and/or treated by several maneuvers, these must be instituted very early after the insult, well before the rise in serum creatinine**(13)**. 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 **(18).**

Interleukin-18 (IL-18), proinflammatory cytokine , is a single, non-glycosylated polypeptide chain containing 157 amino acids. IL-18 was previously known as interferon γ inducing factor (IGIF). This pleiotropic cytokine is produced by monocyte/macrophage cells. Like IL-12, IL-18 plays an important role in cell-mediated immune responses**(19).**

Immunohistochemistry of mouse kidneys demonstrated an increase in IL-18 protein in injured tubular epithelial cells in AKI kidneys compared to normal controls. In a separate study using freshly isolated proximal tubules from mice, it was determined that hypoxic proximal tubules had high levels of IL-18**(13).**

On the basis of the demonstration of IL-18 in injured proximal tubules, IL-18 was measured in the urine. Urine IL-18 was increased in mice with ischemic AKI compared to sham-operated mice. Thus we developed the hypothesis that IL-18 could be released from the injured tubular epithelial cells into the urine and serve as a urinary biomarker of AKI in humans**(14).**

Therefore, the aim of this study is to evaluate urinary IL-18 as early sensitive biomarker of acute kidney injury in critically ill-patients in ICU where their previous serum creatinine is normal. This study was conducted on a total number of 84 patients who were critically ill in ICU. These patients were classified into 2 groups according to occurrence of AKI:

AKI group: includes 36 patients who experienced an elevation of serum creatinine either by (25%) of the basal level or by ≥0.3mg/dl above the basal level after 24 hours of admission.

Non AKI group: includes 48 patients who didn't experienced an elevation of serum creatinine either by (25%) of the basal level or by 0.3mg/dl above the basal level after 24 hours of admission.

The reported incidence of AKI varies widely, ranging from 0% to 50% and this variability results from differences in the presence or absence of risk factors that implicated in AKI **(19).**

About 42 % of the studied patients(36 patients) fulfilled the criteria of AKI .

The current study showed that urinary IL-18(pg/dl) was significantly increased in AKI group after 6 hours compared to its basal level ($p<0.001$). No significant difference was detected between basal and after 6 hours levels of urinary IL-18(pg/dl) in non AKI group ($p>0.05$).

There was no significant difference of Serum creatinine(mg/dl) after 6 hours in both groups compared to their basal values ($p>0.05$). After 24 hours there was further significantly increased of urinary IL-18(pg/dl) in AKI group compared to its basal and 6 hours values ($p<0.001$) ($p<0.001$) respectively. There was no significant difference of urinary IL-18(pg/dl) after 24 hours compared to its basal and 6 hours values in non AKI group ($p>0.05$) ($p>0.05$) respectively.

Serum creatinine(mg/dl) was significantly increased in AKI group after 24 hours compared to its basal and 6 hours values ($p<0.001$)($p<0.01$) respectively There was no significant difference of S.cr.(mg/dl) after 24 hours compared to its basal and 6 hours values in non AKI group ($p>0.05$) ($p>0.05$) respectively.

Our results were supported by **Yawei et al., (2012**) who found that urinary IL-18(pg/dl) was significantly increased in AKI patients after 6 and 24 hours compared to its basal levels ($p<0.001$) ($p<0.001$) respectively in contrary of S.cr.(mg/dl) that was not significantly increased in AKI patients after 6 and 24 hours compared to its basal levels ($p>0.05$) ($p>0.05$) respectively**(20)** .

This increase may be due to damage of tubular epithelial cells or hypoxia in proximal tubules in which IL-18 could be released into the urine.

On the other hand **Wiedemann et al., (2013**) who found no significant difference between 6h urinary IL-18(pg/dl) and its basal values and significant difference between 24h urinary IL-18 (pg/dl) and its basal values in the AKI patients with chronic decompensated heart failure: evidence for cardio renal syndrome **(21).** The difference in results may be due to chronic process of hypoxia to tubular epithelial cells which leads to no change level of urinary IL-18(pg/dl).

This study demonstrated statistical *high significant* correlations between 6h urinary IL-18(pg/dl) and both; basal serum creatinine (mg/dl) and basal urinary IL-18(pg/dl), associated with statistical *significant* correlations with GFR according to MDRD (ml/min) in the AKI group.

This study demonstrated statistical *high significant* correlations between 24h urinary IL-18(pg/dl) and both; basal serum creatinine (mg/dl) and basal urinary IL-18(pg/dl), associated with statistical *significant* correlations with GFR according to MDRD (ml/min) in the AKI group.

There was no statistically significant difference in urinary IL-18 (pg/dl) in the non AKI group at 6h and 24h after admission in ICU .

This results were supported by **Kimberly et al., (2007)** who reported similar correlations showing high significant correlations between 24h urinary IL-18(pg/dl) and both; basal serum creatinine (mg/dl) and basal urinary IL-18(pg/dl), associated with significant correlations with GFR according to MDRD (ml/min) in the AKI group and there was no statistically significant difference in urinary IL-18 (pg/dl) in the non AKI group after 6h and 24h values **(22).**

On the other hand **Wiedemann et al., (2013**) who found no significant correlations between 24h urinary IL-18(pg/dl) and both; basal serum creatinine (mg/dl) and basal urinary IL-18(pg/dl), associated with GFR according to MDRD (ml/min) in the AKI patients with chronic decompensated heart failure: evidence for cardio renal syndrome**(21).**

In our study; There was a highly statistical difference along the time course for urinary IL-18 (pg/dl) and serum creatinine (mg/dl) in patient with AKI and There was no statistical difference along the time course for urinary IL-18 (pg/dl) and creatinine (mg/dl) in patient without AKI.

we found that the ***sensitivity*** and ***specificity*** of urinary IL-18 (pg/dl) at 6h after admission was 91.1% and 93.9% respectively.

These results are in accordance with that obtained by **Bellomo (2013) (12)** who reported similar results in the diagnostic characteristics of different uIL-18 cutoffs to predict AKI development within 24 h. that the ***sensitivity*** and ***specificity*** of urinary IL-18 (pg/dl) at 6h after admission were (91% and 92.3%) (90.5% and 93%) respectively.

On conclusion , Urinary IL-18 (pg/dl) is significantly increased in AKI groups , this increase is earlier than any rise of serum creatinine (mg/dl) in these patients . Urinary IL-18 (pg/dl) is more specific and sensitive in prediction of AKI than serum creatinine (mg/dl) , So urinary IL-18 (pg/dl) can be used as early biomarker of Acute kidney injury in critically ill patients in ICU that may allow early prediction at a time when intensive care optimization can be performed and that can help in prevention of worsening of these cases and good outcome.

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