RESEARCH PAPER INTERNATIONAL JOURNAL OF PURE MEDICAL RESEARCH

Pediatrics

KEYWORDS: Urinary G1, Acute Kidney Injury, TIMP gene

THE CLINICAL USE OF THE URINARY G1 CELL CYCLE ARREST BIOMARKER { TIMP-2} FOR EARLY PREDICTION OF ACUTE KIDNEY INJURY IN CRITICALLY ILL CHILDREN

Volume-3, Issue-7, July - 2018

Mohammed S.Selim	Professor of pediatrics ,Faculty of Medicine , Benha University
Soha A.Ibrahim	Professor of Pediatrics , Faculty of Medicine , Benha University
Ola G.Behairy	Lecturer of Pediatrics , Faculty of Medicine – Benha University
Eman Ramadan	Professor Of clinical pathology, Faculty of Medicine – Benha University
Ghada A.Ibrahim*	M.B.B.Ch Faculty of Medicine - Benha University*Corresponding Author ghada.sharafeldeen@yahoo.com

Article History
Received: 06.02.2018
Accepted: 30.04.2018
Published: 10.07.2018

ABSTRACT.

Background

Acute kidney injury (AKI) is a serious complication, commonly occurring in the critically ill population, with devastating short- and long-term consequences , and its incidence is rising at an alarming rate . Tissue inhibitor of metalloproteinase-2 (TIMP-2) was reported as an emerging biomarker for predicting severe AKI in critically ill patients

Methods

This Prospective case control studywas conducted on 60 critically illchildren of both sex admitted to PICU of Benha children Hospital and 20 healthy control group of both sex. The study was conducted during the period from September 2016 to December 2016 . All cases were followed up for 2 months or until they improved or died. The study group was devided into 3 groups Acute kidney injury group (AKI gp) : critically ill with AKI included 36 patients , Non Acute kidney injury group(Non AKI gp) : critically ill without AKI included 24 patients , Control group : control group comprised 20 apparently healthy children matched in age and sex with patient groups . AKI etiologies were: dehydration/hypovolemia (n = 5) , hemodynamic instability (n = 9), septic shock (n =20) vasculitis (n= 2)

Results

There was statistical significant difference between the studied groups regarding serum TIMP 2 day 1 as it was higher in AKI group than non AKI group than control group

Conclusions

TIMP-2 can be used as a urinary biomarker for early detection of acute kidney injury (AKI) in critically ill patients .

Introduction

Acute kidney injury (AKI) is a serious complication, commonly occurring in the critically ill population, with devastating short- and long-term consequences , and its incidence is rising at an alarming rate . Despite standardization of the definition and staging of AKI, early recognition remains challenging given that serum creatinine level is a marker, albeit imperfect, of kidney function and not kidney injury. Furthermore, the delay in increase in serum creatinine level after loss of glomerular filtration also prevents timely detection of decreased kidney function in patients with AKI . (Vijayan et al ., 2016)

In the past decade, the Kidney Disease Improving Global Outcomes (KDIGO) criteria was validated in large patient cohorts and classifies AKI into three stages based on increases in serum creatinine (sCr) and decreases in urine output (UOP). A recent publication highlighted the importance of UOP in the criteria. The Acute Dialysis Quality Initiative has proposed the inclusion of renal stress/damage biomarkers .(Kellum et al ., 2015) , (Murray et al ., 2014)

Unfortunately, serum creatinine measurement is an imperfect reference standard test both its sensitivity and specificity are affected by age, sex, muscle mass, exercise, and diet, as well as by certain drugs and underlying clinical conditions. In addition, because of kidney reserves, levels of serum creatinine may not increase until as much as 50% of kidney function has been lost.(Malyszko et al.,2014)

Tissue inhibitor of metalloproteinases-2 (TIMP-2) has recently been suggested as a promising tool for the early detection of AKI in critically ill patients . Both proteins are inducers of the G1 cell cycle arrest, considered as a key mechanism of AKI. (Kashani et al ., 2013)

Tissue inhibitor of metalloproteinases-2 (TIMP-2) is a human gene, thought to be a metastases suppressor. This gene is a member of the TIMP gene family. The proteins encoded by this gene family are natural inhibitors of the matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix. In addition to an inhibitory role against metalloproteinases, the encoded protein has a unique role among TIMP family members in its ability to directly suppress the proliferation of endothelial cells. As a result, the encoded protein may be critical to the maintenance of tissue homeostasis by suppressing the proliferation of quiescent tissues in response to angiogenic factors, and by inhibiting protease activity in tissues undergoing remodelling of the extracellular matrix.TIMP-2 functions as both an MMP inhibitor and an activator. TIMPs inhibit all active MMPs, but different TIMPs inhibit different MMPs better than other TIMPs. For example, TIMP-1 inhibits MMP-7, MMP-9, MMP-1 and MMP-3 better than TIMP-2, and TIMP-2 inhibits MMP-2 more effectively than other TIMPs.(Bourboulia et al.,2010)

Recently, tissue inhibitor of metalloproteinase-2 (TIMP-2) was reported as an emerging biomarker for predicting severe AKI in critically ill patients . In cells of various different types, including cells in renal tubules and glomeruli, TIMP-2 is expressed constitutively. Reportedly, TIMP-2 is involved with G1 cell cycle arrest during the early phases of cell injury . Renal tubular cells enter a short period of G1 cell cycle arrest following renal ischemic insult . Therefore, enhanced TIMP-2 expression can be expected in the pathological condition of AKI.(Kashani et al., 2013) , (Meersch., 2014)

Patients and methods :

***Study group :**

This Prospective case control studywas conducted on 60 critically illchildren of both sex admitted to PICU of Benha children Hospital and 20 healthy control group of both sex. The study was conducted during the period from September 2016 to December 2016 . All cases were followed up for 2 months or until they improved or died. The study group was devided into 3 groups :

- Acute kidney injury group (AKI gp) : critically ill with AKI included 36 patients (8 females and 28 males) . 32 patients died and 4 survived . Their mean age was3.33±3.91 ranged0.5-14 yearsdiagnosed by clinical examinations and laboratory investigations using pRIFLE crieteria and GFR by Shwartz formula.

- Non Acute kidney injury group(Non AKI gp) : : critically ill without AKI included 24 patients (7 females and 17 males) . 9 patients died and 5 survived. Their mean age was 5.5 ±5.45 ranged0.17-16 years.

- Control group : control group comprised 20 apparently healthy children matched in age and sex with patient groups and they were clinically free from any diseases collected from vaccination outpatient clinic and from general population.Their mean age 7.53±5.64 ranged 7.53±5.64 ranged 0.08-16 years.

The study was approved by the Ethical Committee of Benha university. An informed consent was obtained from one of the parents before enrollment of the patients.

Inclusion crieteria :
All children who are critically ill and admitted to PICU during the study period .Patients aged from 1 month to 16 years old.

Exclusion crieteria :
Children with chronic kidney disease or any other chronic co-morbidity .

- Age 17 years or older.
- Neonates
- Under chemotherapy

Methods:
All children incorporated in this study were subjected to the following:

A-Careful history taking regarding:
* Personal history : Name, age, sex, residence and social level.
* Complaint.
* History of present illness.
*Past history of diseases, operations or medication.
*Family history.
*Dietetic and vaccination history.

B-Thorough clinical examination regarding
- General examination including

1-level of consciousness and complexion (pallor, cyanosis).

2 -Vital signs (heart rate. respiratory rate, blood pressure and temperature)

3-Anthropometric measurements: including weight and height were recorded. Body mass index (BMI) was calculated as kg/m2 (Normal BMI = 18.5–24.9, underweight = BMI <18.5 and Overweight BMI = 25–29). Weight was measured in kg (to the nearest 100 grams) using an electronic digital scale and its accuracy was periodically verified using reference weights. Length was measured in cm (measured to the nearest mm); children were measured on scales with height gauges, the subject standing with back against the gauge and feet on the weighing platform. All measurements were taken by the same person.Patients' height & weight for age percentiles were checked according. (WHO, 2006)

Systemic examination
1. Chest examination
- Inspection for: retractions, chest movements, and signs of respiratory distress).
- Palpation for tracheal shift and palpable bronchi.
- Auscultation for (breath sounds and adventious sounds).

2. Cardiac examination
Inspection and papation : for pericordialpulge,pulsations and thrill
Auscultation : for s1, and s2 and for murmurs

3. Neurological examination
- Glasgow Coma Scale was done, examination for motor, sensory, cerebellar and extrapyramidal systems.
- Pupil examination for equality and reactivity was done.

4. Abdominal examination
- Inspecion : for abdominal enlargement, stria,scratch marks and pigmentation
- Palpation : for organomegally
- Auscultation : for intestinal sounds
- Percussion : for ascitis
- Scoring procedure

The severity of illness in the first 24 hours was assessed as defined by Pediatric Risk of Mortality III (PRISM-III) score.

In brief, PRISM III was scored based on age related physiological parameters including systolic blood pressure, heart rate, temperature, pupillary reflexes, mental status, acidosis (pH and total Co2), pCO2, pO2, glucose, potassium, creatinine, blood urea, white blood cell count, platelet count, and prothrombin or partial thromboplastin time.

Total PRISM III score = (cardiovascular and neurologic subscore) + (acid base and blood gas subscore) + (chemistry subscore)+ (hematology subscore)

Interpretation:
• minimumsubscore and total score: 0
• maximum cardiovascular and neurologic subscore: 30
• maximum acid-base and blood gas subscore: 22
• maximum chemistry subscore: 10
• maximum hematology subscore: 12
• maximum total PRISM III score: 74
• The higher the total score, the worse the prognosis.
• A rising score indicates deterioration.
(Bhadoria and Amit.,2008)

Laboratory Investigation:
- 1 ml of heparinized sterile arterial blood was taken for ABG and was done using 9180 Electrolyte Analyzer.

- six ml venous blood was collected from each subject by clean venipuncture using a disposable plastic syringe and subsequently divided into:

- 2 ml of blood was taken on ethylene diamine tetra-acetic salt (EDTA) (1.2mg/mL) as an anticoagulant, then was used for complete blood count (CBC) Done by automated hematology analyzer sysmexxs 800 (SN:63387) (Tatsumi et al , 2002).

- 2 ml of blood was taken on sodium citrate, as an anticoagulant and was used for measurement of PT,PTT Done by CoaDATA4004. (Levy et al.,2014)

- 2ml of blood was taken on a plain tube (without anticoagulant) for serum separation. The tube was left at room temperature for 30 minutes till coagulation, and then was centrifuged (at 1500

rpm for 15 minutes). The resultant serum was used for clinical chemistry tests: CRP , Urea, creatinine , potassium and blood sugar. Done by BT3500.

- GFR was calculated by Scwartz formula at day 1 and 3.
- Urine output : was calculated on day 1 and 3 by assembling the total amount of urine excreted in 24 hours divided by24 then devided by the patient's weight
- Urine analysis : 10 ml of fresh urine was collected in a clean jar then was sent to the laboratory to be examined for glucose , acetone and protein .

$$\text{eGFR} = \frac{k \times Height}{Serum\ Creatinine}$$

Biomarker Assay (TIMP-2)

1ml of freshly obtained urine sample Centrifuged for 20 minutes at 1000×g at 2 - 8°C. The supernatant was collected and the assay was carried out immediately. Done by using Human TIMP-2 (Metalloproteinase inhibitor 2) ELISA kit manufactured and distributed by USA and Canada and using plate washer V-230 A 0.35 model TIPO D1 and plate reader V230 A 0.3 model TIPO A4.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- TIMP-2antibody was pre-coated onto 96-well plates. And the biotin conjugated anti- TIMP-2 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the TIMP-2 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of TIMP-2 can be calculated.(Li et al., 2001)

Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages using Chi square test (X2) and Fisher's exact test to analyze them. Continuous variables were tested for normality using Kolmogorov Smirnov test, Quantitative data were expressed as mean ± standard deviation and range if normally distributed, using Student "t" test to compare means of 2 parametric variables. ANOVA test was used to compare 3 parametric independent variables. Non parametric variables were expressed as median and IQR using Man Whitney U test (MWU) for two variables or Krauskal Wallis test (KWT) 3 independent variables. Spearman's correlation coefficient (rho) was used to test correlations between non parametric variables. Receiver Operator Characteristic curve (ROC curve) was used to detect the cutoff value of vitamin D level with optimum sensitivity and specificity in the prediction of DM and its control state. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

Results:

Eighty subjects were enrolled in the study including 36 AKI patients, 24 patients without AKI (non-AKI group) and 20 apparently healthy control children . The epidemiological data are presented in table (1). In table (1) , there was statistical significant difference between the studied groups regarding age , weight and height as it was higher in control group than non AKI group than AKI group.However ,there was no statistical significant difference regarding sex and BMI.

Table(1) : comparison between the studied groups regarding sociodemographic data

Variable		AKI (no.=36)		Non-AKI (no.=24)		Control (no.=20)		Test	P
		No.	%	No.	%	No.	%		
Sex	Female	8	22.22	7	29.17	6	30.0	X^2= 0.55	0.76
	Male	28	77.78	17	70.83	14	70.0		
		Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
Age (years)		3.33±3.91	0.5-14	5.54±5.45	0.17-16	†7.53±5.64	0.08-16	F= 4.96	0.009 (S)
Weight (kg)		16.54±13.66	9-54	17.5±9.03	3-33	†26.37±16.63	3-50	F= 3.69	0.03 (S)
Height (cm)		83.97±18.31	70-134	†91.67±27.88	43-134	†112.7±35.7	50-160	F= 7.67	<0.001 (HS)
BMI (kg/m^2)		20.78±6.3	15-37.5	19.51±4.09	10.2-26.6	18.52±3.21	12-23.14	Kruskal Wallis test= 0.04	0.98

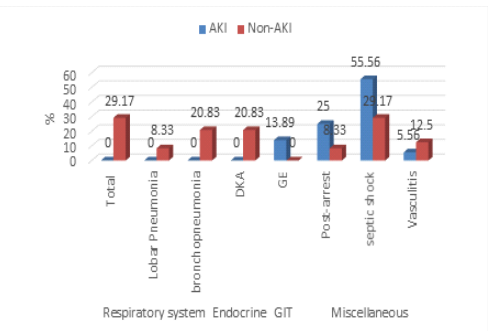
F: OnewayAnalysisOf Variance (ANOVA) ,X2: Chi-squared test , S: Significant (P<0.05) , HS: Highly Significant (P<0.001) , †: Significant differences compared to AKI group

As regarding AKI etiologies : AKI etiologies were: dehydration/hypovolemia (n = 5) , hemodynamic instability (n = 9), septic shock (n =20) vasculitis (n= 2) as in table (2) & Fig (1)

Table(2):comparison between the studied patient groups as regard diagnosis:

Diagnosis		AKI (no.=36)		Non-AKI (no.=24)		Z	P
		No.	%	No.	%		
Respiratory system	Total	0	0.0	7	29.17	3.45	<0.001 (HS)
	Lobar Pneumonia	0	0.0	2	8.33	1.76	0.09
	bronchopneumonia	0	0.0	5	20.83	2.86	0.004 (S)
Endocrine	DKA	0	0.0	5	20.83	2.86	0.004 (S)
GIT	GEdehydration/hypovolemia	5	13.89	0	0.0	1.91	0.06
Miscellaneous	Post-arrest hemodynamic instability	9	25.0	2	8.33	1.63	0.10
	septic shock	20	55.56	7	29.17	2.01	0.04 (S)
	Vasculitis	2	5.56	3	12.50	0.95	0.34

Z: the test of proportion ,S: Significant (P<0.05) , HS: Highly Significant (P<0.001)



There was statistical significant difference between the studied patient groups regarding PRISM III score which is higher in AKI group than non AKI group .Also regarding pRIFLEcrieteria it was higher regarding Injury and Failure in AKI group than non AKI group. Table (3) & Fig (2 , 3)

Table (3):comparison between the studied patient groups regarding PRISM III score and pRIFLE:

Variable		AKI (no.=36)		Non-AKI (no.=24)		Test	P
		Mean ±SD	Range	Mean ±SD	Range		
PRISM III day1		16.72±5.6	3-25	5.58±10.52	1-30	Z= 4.52	<0.001 (HS)
pRIFLE	0, n(%)	0 (0.0)		24 (100))		FET	<0.001 (HS)
	Risk ,n (%)	0 (0.0)		0 (0.0)			
	Injury , n (%)	30 (83.33)		0 (0.0)			
	Failure , n (%)	6 (16.67)		0 (0.0)			
	Loss of function ,n(%)	0 (0.0)		0 (0.0)			

Z: Mann-Whitney test ,HS: Highly Significant (P<0.001) , FET: Fisher Exact Test

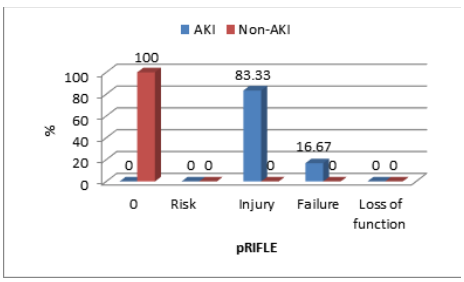


Figure (2) : Means of pRIFLE

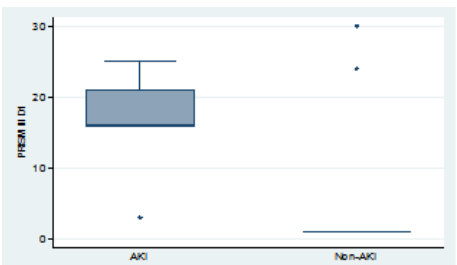


Figure (3) : Means of PRISM III on day 1

That there was statistical significant difference between the studied patient groups regarding death and the cause of death as death rate was higher in AKI group 88.89% to 37.5%, and the cause of death due to sudden cardiorespiratory collapse was higher in non AKI group as it was 37.5% to 2.78% and arrhythmia was higher in AKI group 77.78%. Table (4) & Figure (4)

Table (4) :Comparison between the studied patient groups regarding treatment of AKI , death and cause of death :

Variable		AKI (no.=36)		Non-AKI (no.=24)		Test	P
		No.	%	No.	%		
Treatment of AKI	Conservative	34	94.44	-	-	-	-
	Haemodialysis	2	5.56	-	-		
Death	No	4	11.11	15	62.5	X^2= 17.57	<0.001 (HS)
	Yes	32	88.89	9	37.5		
Cause of death	Arrhythmia	28	77.78	0	0.0	Z= 5.92	<0.001 (HS)
	Severe ICH	3	8.33	0	0.0	Z= 1.45	0.15
	Sudden cardiorespiratory collapse	1	2.78	9	37.50	Z= 3.53	<0.001 (HS)

X2: Chi-squared test ,Z: the test of proportion , HS: Highly Significant (P<0.001)

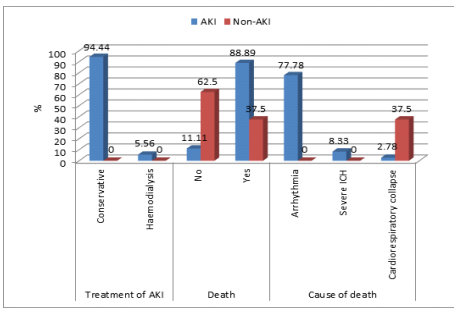


Figure (4) : Means of treatment of AKI , death and cause of death

There was statistical significant difference between the patient groups regarding length of PICU stay and length of hospital stay as it was higher in non AKI group than AKI group . Table (5)

Table (5):Length of PICU and hospital stay in patient groups (AKI group and Non AKI group):

Variable	AKI (no.=36)		Non-AKI (no.=24)		T	P
	Mean ±SD	Range	Mean ±SD	Range		
Length of PICU stay (days)	7.03±7.01	3-22	14.08±7.45	3-24	3.72	<0.001 (HS)
Length of hospital stay (days)	7.11±7.19	3-23	14.21±7.35	3-24	3.71	<0.001 (HS)

t: Student t-test , HS: Highly Significant (P<0.001)

F: Oneway Analysis Of Variance (ANOVA) ,X2: Chi-squared test , S: Significant (P<0.05) , HS: Highly Significant (P<0.001) , †: Significant differences compared to AKI group , ‡: Significant differences compared to non-AKI group

There was statistical significant difference between the studied groups regarding TLC and CRP as it was higher in AKI group than non AKI group than control group. Also regarding Hb and PLT was lower in AKI group than non AKI group than control group . Table (6) & Figure (5,6,7,8)

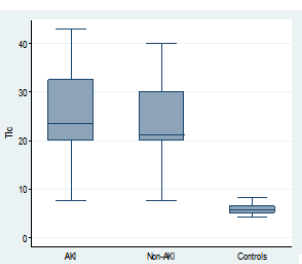


Figure (5) :Means of TLC

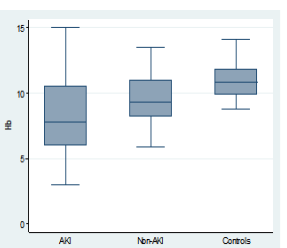


Figure (6) : Means of Heamoglobin

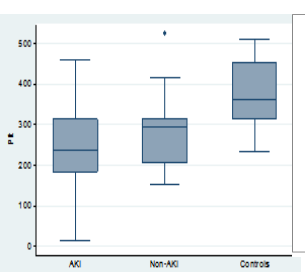


Figure (7) : Means of Platelets

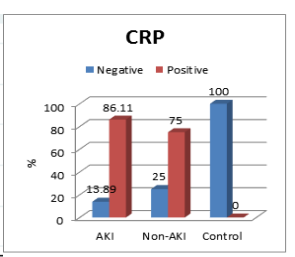


Figure (8) : Means of CRP

Table(6): Comparison between the studied groups regarding CBC and CRP :

Variable		AKI (no.=36)		Non-AKI) (no.=24)		Control (no.=20)		Test	P
		Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
TLC		26.55±9.11	7.6-43	23.42±8.14	7.6-40	††5.81±1.14	4.2-8.2	F= 50.49	<0.001 (HS)
HB (gm/dl)		8.5±2.89	3-15	9.53±1.95	5.9-13.5	†10.95±1.45	8.8-14.1	F= 7.14	0.001 (S)
PLT		244.72±109.02	15-460	285.5±88.71	154-525	††374.35±80.39	233-512	F= 11.58	<0.001 (HS)
CRP	Negative n (%)	5 (13.89)		6 (25.0)		20 (100.0)		X^2=42.90	<0.001 (HS)
	Positive n (%)	31 (86.11)		18 (75.0)		0 (0.0)			

There was statistical significant difference between the studied groups regarding serum Creatinine in day 3 , serum Urea day 1 , serum Potassium and TIMP 2 day 1 as it was higher in AKI group than non AKI group than control group. Also regarding UOP on day 1 and 3 and GFR on day 1 and 3 which was higher in control group than non AKI group than AKI group. As regarding protein in urine and protein creatinine ratio was highly significant in AKI group . Table (7) & Figure (9,10,11,12,13,14)

Table(7)comparison between the studied groups regarding renal function tests :

Variable		AKI (no.=36)		Non-AKI (no.=24)		Control (no.=20)		Test	P
		Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
Creatinine day1 (mg/dl)		1.32±0.44	0.64-1.7	1.3±0.24	0.4-1.7	1.2±0.51	0.3-1.5	0.53	0.59
Creatinine day3 (mg/dl)		3.14±0.46	2-4.9	†0.85±0.22	0.4-1.05	††0.47±0.1	0.3-0.65	F= 540.20	<0.001 (HS)
Urea day1 (mg/dl)		85.78±41.21	35-286	†54.25±17.55	17-68	††29.25±7.16	20-40	Kruskal Wallis test= 47.20	<0.001 (HS)
Urine analysis	Normal Urine analysis (%)	18 (50.0)		19 (79.17)		20 (100.0)		FET <0.001 (HS)	
	Glucose ++/Acetone ++, n (%)	0 (0.0)		5 (20.83)		0 (0.0)			
	Ptn.+, n (%)	18 (50.0)		0 (0.0)		0 (0.0)			
	Prt/Cr. Ratio	8.98±9.15	0-21.4	†0	0	†0	0	Kruskal Wallis test= 14.67	<0.001 (HS)
UOP day1 (ml/Kg/h)		2.12±0.33	1-2.7	†2.66±0.76	2-5	†2.44±0.41	2-3.1	Kruskal Wallis test= 15.93	<0.001 (HS)
UOP day3 (ml/Kg/h)		0.42±0.16	0.1-0.7	†2.4±0.43	2-3.3	†2.44±0.41	2-3.1	F=360.72	<0.001 (HS)
Serum potassium (mmol/L)		6.94±0.7	5.7-8	†3.83±0.64	3.2-5.5	†4.3±0.7	3.5-5.5	F= 180.57	<0.001 (HS)
TIMP2 day1 (ng/ml)		14.23±1.37	11.9-17.76	†5.08±3.34	0.01-10.5	††1.33±1.37	0.15-4	F= 267.72	<0.001 (HS)
GFR day1 (ml/min/1.73m²) By Scwartz formula		38.76±29.67	18.5-133	61.37±42.17	26-156	††141.4±70.88	97-171	F=31.96	<0.001 (HS)
GFR day3 (ml/min/1.73m²) By Scwartz formula		15.06±6.66	9.5-42	†67.48±39.89	31.5-156	††141.4±70.88	97-171	F=59.39	<0.001 (HS)

F: Oneway Analysis Of Variance (ANOVA) ,Z: Mann-Whitney test , FET: Fisher Exact Test , t: Student t-test

HS: Highly Significant (P<0.001) ,†: Significant differences compared to AKI group , ‡: Significant differences compared to non-AKI group

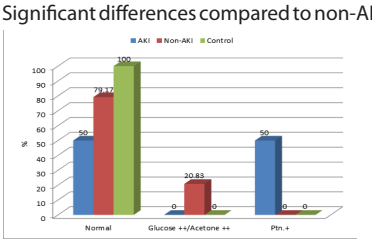


Figure (9) : Means of Glucose , Acetone and Protein in urine

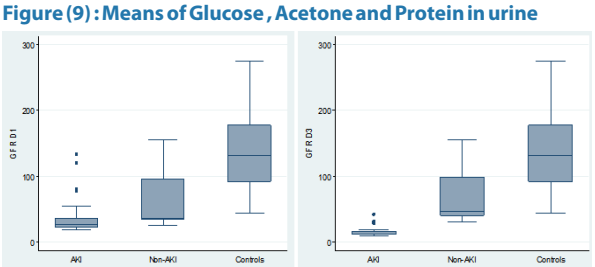


Figure (10) : Means of GFR on day 1
Figure (11) : Means of GFR on day 3

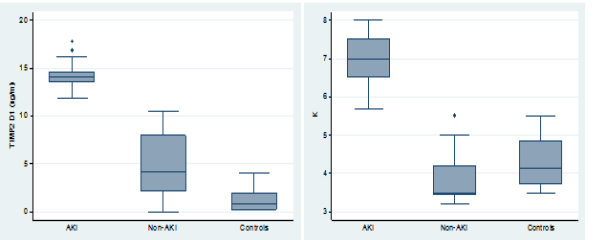


Figure (12) : Means of TIMP-2 on day 1
Figure (13) : Means of serum Potassium level

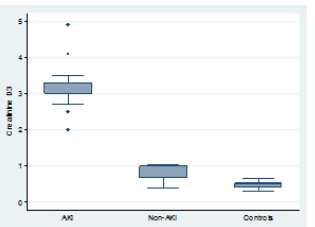


Figure (14) : Means of serum Creatinine level on day 3

There was statistical significant difference in AKI group regarding treatment of AKI whether conservative or by heamodialysis and death . There is no statistical difference regarding sex , CRP , urine analysis and pRIFLE . Table(8)

There was negative correlation regarding age , Hb , GFR day1 and GFR day 3 in non AKI group and there was positive correlation regarding PRISM III , Creatinine on day 1 and day 3 and Urea on day 1 in non AKI group. Table (9)

The cutoff value , regarding Diagnostic performance of TIMP2 for early detection of AKI in cases , is11.9 , sensitivity is 100% , specificity is 100% , PPV 1s 100% , AUC is 1 , correctly diagnosed cases are 100% . Table (10) & Fig (15)

Regarding diagnostic performance of TIMP-2 level for death in AKI group , the cutoff value is 16.8 with sensitivity 90.62% , Specificity 25% , PPV 90.62% , AUC 0.773 , correctly diagnosed cases 83.33% . Table (11) & Fig (16) Regarding Diagnostic performance of TIMP2 for hemodialysis in AKI cases , the cutoff value is 16.8 with sensitivity 50% , Specificity 100% , PPV 100% , AUC 0.92 , correctly diagnosed cases 97.2% . Table (12) & Fig (17)

Table (8): comparison between TIMP-2 levels in AKI group regarding Sex , CRP , Urine analysis , pRIFLE , treatment of AKI , Death :

Variable (no.=36)		TIMP2			T	P
		No.	Mean ±SD	Range		
Sex	Female	8	14.19±0.47	13.3-14.6	0.09	0.93
	Male	28	14.24±1.54	11.9-17.76		
CRP	Negative	5	14.69±1.83	13.3-17.76	0.81	0.42
	Positive	31	14.15±1.3	11.9-16.8		
Urine analysis	Ptn.	18	14.6±1.48	12.2-17.76	1.66	0.11
	Normal urine analysis	18	13.86±1.18	11.9-16.8		
pRIFLE	Failure	6	14.5±1.06	13.7-16.2	0.53	0.60
	Injury	30	14.17±1.43	11.9-17.76		
Treatment of AKI	Conservative	34	14.1±1.25	11.9-16.8	2.51	0.02 (S)
	Haemodialysis	2	16.43±1.88	15.1-17.76		
Death	No	4	15.52±1.34	13.7-16.8	2.11	0.04 (S)
	Yes	32	14.06±1.3	11.9-17.76		

: Student t-test , S: Significant (P<0.05)

Table (9) : correlation between TIMP-2 and estimated parameters:

Variable	AKI (no.=36)		Non-AKI (no.=24)		Control (no.=20)	
	R	P	R	P	r	P
Age (years)	0.27	0.11	**-0.48**	**0.02 (S)**	-0.07	0.78
Weight (kg)	0.31	0.06	-0.36	0.09	-0.11	0.65
Height (cm)	0.21	0.22	-0.31	0.14	-0.07	0.77
BMI (kg/m²)	...	0.18	ρ=0.33	0.11	ρ=-0.04	0.85
Length of PICU stay (days)	0.18	0.30	0.38	0.06	-	-
Length of hospital stay (days)	0.18	0.28	0.37	0.08	-	-
PRISM III day1	ρ=-0.23	0.18	...	**0.01 (S)**	-	-
TLC	0.06	0.70	0.32	0.13	-0.07	0.78
HB (gm/dl)	0.24	0.16	**-0.44**	**0.03 (S)**	0.04	0.88
PLT	0.18	0.30	-0.18	0.38	0.19	0.43
Creatinine day1	-0.07	0.69	**0.71**	**0.001 (S)**	0.33	0.16
Creatinine day3	0.03	0.84	**0.76**	**<0.001 (HS)**	0.33	0.16
Urea day1	ρ=-0.06	0.71	**ρ=0.80**	**<0.001 (HS)**	ρ= -0.005	0.98
Prt/Cr. Ratio	ρ=-0.30	0.07	-	-	-	-
UOP day1	ρ=0.16	0.36	ρ=-0.22	0.30	ρ=0.08	0.73
UOP day3	0.18	0.30	-0.15	0.48	0.13	0.58
K	-0.09	0.59	0.22	0.29	-0.37	0.10
GFR day1	0.21	0.23	**-0.54**	**0.007 (HS)**	-0.27	0.25

r: Pearson correlation coefficient , ρ: Spearman correlation coefficient (rho) , S: Significant (P<0.05) , HS: Highly Significant (P<0.001)

Table (10)Diagnostic performance of TIMP2 for early detection of AKI in cases:

Sample	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	Correctly diagnosed (%)
Cases (no.=60)	11.9	100.0	100.0	100.0	100.0	1.00	100.0

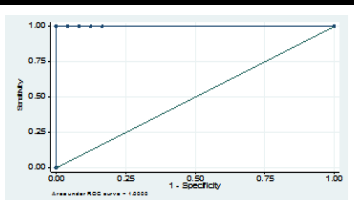


Figure (15) shows Diagnostic performance of TIMP2 for early detection of AKI in cases

Table (11) shows diagnostic performance of TIMP-2 level for death:

Sample	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	Correctly diagnosed (%)
AKI (no.=36)	16.8	90.62	25.0	25.0	90.62	0.7773	83.33

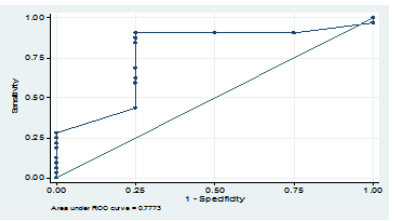
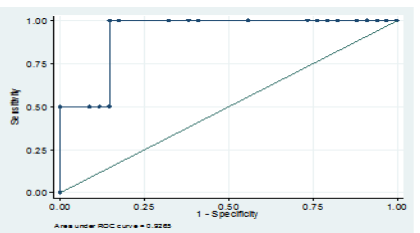


Figure (16) shows diagnostic performance of TIMP-2 level for death in AKI group:

Table(12):Diagnostic performance of TIMP2 for hemodialysis in AKI cases

Sample	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	Correctly diagnosed (%)
Cases (no.=36)	16.8	50.0	100.0	100.0	97.14	0.9265	97.22



Figure(17):Diagnostic performance of TIMP2 for hemodialysis in AKI cases

Discussion:

Acute kidney injury (AKI) in pediatric patients is characterized clinically by rapid loss of glomerular filtration rate (GFR), resulting in a failure to excrete end products of nitrogen metabolism and to maintain fluid volume, electrolyte and acid-base homeostasis .(Vanmassenhove et al., 2013)

The incidence of AKI in hospitalized children is rising, and the etiology of pediatric AKI (pAKI)is dramatically changing from isolated acute renal disease to multiple organ failure. Using pediatric-modified Risk, Injury, Failure, Loss, End-Stage Kidney Disease (pRIFLE) criteria, the incidence of AKI varies between 30 % and 50 % in children undergoing cardiac surgery for congenital heart disease and may jump to extreme rates (up to 82 %) in critically ill children with multiple organ failure .(Vanmassenhove et al ., 2013)

Acute kidney injury (AKI) has a high impact on health care systems because of its high morbidity and mortality rates,length of hospital stay, and treatment costs. Thus, prevention and early diagnosis are essential to provide measures to avoid the onset of dialysis as much as possible. Although molecular markers of early kidney damage would be ideal, they are unfortunately unavailable for routine clinical use.Therefore, variations in serum creatinine according to the Acute Kidney Injury Network (AKIN) and Kidney Disease Improve Global Outcome (KDIGO) criteria remain a valid tool for diagnosis .(Remuzzi and Horton , 2013)

Extensive research efforts over the last decade have been directed at the discovery and validation of numerous (>28) novel AKI biomarkers. Most of these protein-bound biomarkers are indicators of structural renal damage rather than of decreased kidney function. An ideal AKI biomarker should be accurate, reliable, easy to measure with a standard assay, noninvasive, reproducible and sensitive, and specific with defined cutoff values. Of great importance, novel biomarkers of AKI must provide additional information that is not surmised from clinical evaluation and standard laboratory tests. Urine represents an ideal body fluid for AKI biomarker assessment, as it can be obtained non invasively and

repeatedly from a spontaneously voided urine sample orfrom an indwelling bladder catheter. Urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL),kidney-injury molecule-1 (KIM-1), interleukin-18 (I-18),urinary liver-type fatty-acid-binding protein (L-FABP) , tissue inhibitor of metalloproteinase 2(TIMP-2) and insulin like growth factor binding protein 7 (IGFBP 7)show promise in both diagnostic and prognostic utility in patients with AKI arising from various causes .(Kimmel et al ., 2016

Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) is a protein expressed by healthy human kidneys that contribute to a cell-cycle arrest of the tubular cells in the kidneys in response to injury .(Konvalinka , 2014)

A few studies have shown that tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) are specific biomarkers of structural renal damage in critically ill patients. Renal tubular cells enter in G1 cell arrest to block the effects of molecules contributing to cell-cycle promotion, such as cyclins. This mechanism prevents the extension of cell damage. TIMP-2 and IGFBP7 are protective molecules involved in G1 cell-cycle arrest that moderate apoptotic, angiogenic, inflammatory and ischemic processes. Since renal cell arrest usually occurs 24–48 h before sCr rises due to a significant fallin the glomerular filtration rate, therefore enhanced TIMP-2 expression can be expected in the pathological condition of AK and thought to be earlier AKI biomarkers than sCr. (Hoste et al., 2014).

So the aim of our work was to assess the clinical use of TIMP-2 level as a urinary biomarker for early detection of acute kidney injury (AKI) in critically ill children.

In the current study, there was statistical significant difference between the patient groups regarding length of PICU stay, length of hospital and PRISM stay as it was higher in non AKI group than AKI group.While PRISM III score was higher in AKI group than non AKI grouped. Our results were in agreement with Westhoff et al ., 2015, who found that The length of the ICU stay was 4-fold and the length of hospitalization 1.7-fold higher in the AKI group compared to the non-AKI group.Also Dong et al., 2017, found that lengths of hospital stay were longer in AKI group than non-AKI groups

But these results disagreement with Schanzet al., 2017 who studied the predictive ability of urinary [TIMP-2] for development of AKI in decompensated heart failure on 400 patients and found that Patients with AKI stage 2–3 had a significantly longer hospital stay than those with no or stage 1 AKI (P = 0.005).Forty patients had ADHF upon presentation and sufficient data for AKI staging. 27.5%developed AKI stage 2–3 within 7 days.

Also Meersch et al., 2014 and Gist et al., 2017, found that length of stay in the intensive care unit and hospital, respectively, were not different among patients with and without AKI .

In the present study there were statistically significant lower values of hemoglobin in AKI group than non AKI group than controls.Also there was statistical significant difference between the studied groups regarding baseline serum Creatinine in day 1 and 3, serum Urea day 1, protein in urine and protein creatinine ratio, serum Potassium and TIMP 2 day 1 were found significantly higher in AKI group than non AKI group than the control group. However regarding urine output (UOP) on day 1 and 3 and GFR on day 1 and 3 which was higher in control group than non AKI group than AKI group. Our results were in agreement with Westhoffet al ., 2015who found that SCr on study enrollment was significantly higher in the AKI group compared to the non-AKI group. In addition, the AKI group displayed significantly higher values for urinary protein concentration and urinary protein-to-creatinine ratio compared to the non-AKI group(P < 0.001) .Also regarding Median urinary [TIMP-2] not significantly (P = 0.41) higher in the non-AKI group II compared to the non-AKI group I but significantly increased in the AKI group (P<0.001 vs. non-AKI group I; P = 0.001 vs. non-AKI-group II).

Gist et al., 2017 found that Urinary TIMP-2 was significantly higher in patients with AKI and increased by a median of 12 hours (IQR 6–16 hours) before a rise in serum creatinine in patients with all stages of AKI was detectable. Including those patients with stage 2 or 3 AKI, TIMP-2 increased by a median of 13 hours (IQR 9–17 hours) prior to a rise in serum creatinine.

Meersch et al., 2014, found that AKI occurred in 12 (24%) of the 51 patients 1.67 days after surgery. Inpatients who developed AKI, SCr significantly increased from0.3 mg/dl (SD 0.1) at baseline to 0.7 mg/dl (SD 0.2) two days after surgery, whereas SCr in patients who did not develop AKI remained unchanged. In the 39 patients who never developed AKI, a significant decrease in urinary [TIMP-2] level after surgery was noted as compared to the preoperative measurement (p,0.01). By contrast, those who subsequently developed AKI had a striking rise in urinary [TIMP-2] level 4 h after the procedure as compared to the pre-surgery values.

In the current study regarding comparison between TIMP-2 levels in AKI and non- AKI group there was statistical significant difference in AKI group regarding treatment of AKI whether conservative or by hemodialysis and death. However there was no statistical significant difference in AKI and non AKI group regarding sex, CRP, urine analysis .Regarding correlation between TIMP-2 and estimated parameters in both AKI and non- AKI group there was negative correlation regarding age, Hb, GFR day1 and GFR day 3 in non AKI group and there was positive correlation regarding PRISM III,Creatinine on day 1 and day 3 and Urea on day 1 in non AKI group. There was no statistical significant correlations between TIMP-2 and estimated parameters in AKI group.

In the present study multiple linear regression analysis of TIMP-2 strengthened the ability to identify those at risk for AKI when added top RIFLE, PTN/Cr ratio, creatinine day 1, GFR day1,GFRday3 and PRISM III.ROC curve analysis for the accuracy of [TIMP-2] in prediction of AKI revealed the cutoff value is 11.9 with sensitivity 100.0% , Specificity 100.0% , PPV 100.0% , NPV 100.0%, AUC 1.00 and for detection of hemodialysis (renal replacement therapy [RRT])revealed the cutoff value is 16.8 with sensitivity 50.0% , Specificity 100.0% , PPV100.0% , NPV 97.14% , AUC 0.926.

In Cuartero et al ., 2017study ,The AUROC curve of the worst value TIMP-2within the first 12 h of ICU admission for AKI prediction was 0.798 , with sensitivity of 73.5% and specificity of 71.4% .

Also as regard diagnostic performance of TIMP 2 , several studies were done as following :

- Hoste , 2014 : AUC was 0.79 , sensitivity 89% and specificity 53%
- Meersch , 2014 : AUC was 0.84 , sensitivity 92% and specificity 81%.
- Bihorac, 2014 :AUC was 0.82 , sensitivity 92% and specificity 46%. They enrolled 408 critically ill patients from both surgical and medical ICUs .71 patients developed AKI, 337 didn't develop AKI and 12 samples were either missed or invalid. Most of the patients presented with acute cardiovascular or respiratory dysfunction and sepsis at ICU admission (79, 70, and 24%, respectively).
- Wetz , 2015 : AUC was 0.70 , sensitivity 47% and specificity 96% . 42 patients were included in the study Sixteen patients (38%) experienced AKI within 60 hours postoperatively, whereas twenty-six patients (62%) had no AKI. Thirteen patients (31%) were classified as KDIGO 1 and three patients (7%) as KDIGO 2. KDIGO 1 was achieved due to serum creatinine rise in seven patients, and six patients had primarily oliguria. KDIGO 2 was classified twice on the basis of oliguria, and one patient showed

an increase of serum creatinine

- Pilarczyk , 2015 : AUC was 0.86 , sensitivity 80% and specificity 81% . This was prospective cohort study done on 60 consecutive patients 48 male and 12 female patients with 19 patients developed an AKI (31.6 %), six patients met the endpoint with AKI 2 or 3 (10 %).

- Dusse , 2016 : AUC was 0.97 , sensitivity 100% and specificity 90%

- Kimmel , 2016 : AUC was 0.76 , sensitivity 76% and specificity 53% . Of 400 enrolled patients, 298 had sufficient sCrandUOdata for classification by KDIGO AKI criteria:AKI stage 2 developed in 37 patients andAKI stage 3 in nine patients.All urinary biomarkers, sCr, and plasma cystatin C had statistically significant (P,0.05) odds ratios (ORs) for the AKI end point.

- Gunnerson , 2016 : AUC was 0.84 , sensitivity 89% and specificity 49%. A total of 375 patients were included in the final analysis of whom 35 (9%) developed moderate-severe AKI within 12 hours .

- Honore , 2016 : AUC was 0.84 , sensitivity 77.5% and specificity 75%. They included 232 patients in the analysis and 40 (17%) developed acute kidney injury. We observed significantly higher urine tissue inhibitor of metalloproteinase-2 in patients with acute kidney injury than without acute kidney injury in both patients with low and high non-renal Sequential Organ Failure Assessment scores ($p < 0.001$).

Limitations of the study:

- The main problem is the size of the sample and the difference in the ages of the patients

- Also Schwartz formula based on serum creatinine and height were considered the gold standard despite the controversy and it is used in clinical practice.

- Another drawback was that the unavailability of hemodialysis and lacking experience of peritoneal dialysis.

Conclusions :

- Tissue inhibitor of metalloproteinases-2 (TIMP-2) has a good diagnostic and prognostic performance in pediatric patients admitted in PICUs and developed AKI .

- TIMP-2 can be used as a urinary biomarker for early detection of acute kidney injury (AKI) in critically ill patients .

- The [TIMP-2] test allowed us to identify patients at increased AKI risk.

- Despite the aforementioned limitations, the results of this metaanalysis indicated that urinary [TIMP-2]·[IGFBP7] may be a reliable biomarker for the early detection of AKI. However, given the significant heterogeneity among the included studies, clinicians should be aware of the utility and limitations of this biomarker in clinical practice.

Recommendations :

- The use of TIMP-2 as screening tests for early detection of kidney injury.

- Further studies to estimate the reference range for TIMP-2 in Egyptian pediatric intensive care units.

- New studies with a bigger sample of patients and more accurate gold standards to use for comparisons will be necessary to establish TIMP-2 as biochemical markers for monitoring GFR in unstable critically ill children.

- New studies should be applied on sick neonates admitted to NICUs .

REFERENCES :

1. Bihorac A, Chawla LS, Shaw AD, Al-Khafaji A, Davison DL, Demuth GE, Fitzgerald R, Gong MN, Graham DD, Gunnerson K, Heung M, Jortani S, Kleerup E, Koyner JL, Krell K, Letourneau J, Lissauer M, Miner J, Nguyen HB, Ortega LM, Self WH, Sellman R, Shi J, Straseski J, Szalados JE, Wilber ST, Walker MG, Wilson J, Wunderink R, Zimmerman J, Kellum JA. Validation of cell-cycle arrest biomarkers for acute kidney injury using clinical adjudication. Am J Respir Crit Care Med. 2014;189(8):932–939. doi: 10.1164/rccm.201401-0077OC.
2. Bourboulia D, Stetler-Stevenson WG (Jun 2010). "Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): positive and negative regulators in tumor cell adhesion". Seminars in Cancer Biology. 20 (3): 161–8.doi:10.1016/j.semcancer.2010.05.002.PMC 2941566.PMID 20470890
3. Cuartero M , Ballús J, Sabater J, Pérez X, Nin N, Ordonez-Llanos J, Betbesé AJ . Cell-cycle arrest biomarkers in urine to predict acute kidney injury in septic and non-septic critically ill patients. Ann Intensive Care. 2017 Sep 7;7(1):92. doi: 10.1186/s13613-017-0317-y.
4. Dong L . , Ma Q . , Bennett M . , Devarajan P . , (2017) : Pediatr Nephrol. 2017 Dec;32(12):2351-2360. doi: 10.1007/s00467-017-3748-7. Epub 2017 Jul 28.PMID:28755073
5. Gist KM, Goldstien SL, Wrona J, Alten JA, Basu RK, Cooper DS, Soranno DE, Duplantis J, Altmann C, Gao Z, Faubel S. Kinetics of the cell cycle arrest biomarkers (TIMP-2*IGFBP-7) for prediction of acute kidney injury in infants after cardiac surgery. Pediatr Nephrol. 2017 Apr 5. doi: 10.1007/s00467-017-3655-y.
6. Honore PM, Nguyen HB, Gong M, Chawla LS, Bagshaw SM, Artigas A, et al. Urinary Tissue Inhibitor of Metalloproteinase-2 and Insulin-Like Growth Factor-Binding Protein 7 for Risk Stratification of Acute Kidney Injury in Patients with Sepsis. Critical Care Medicine. 2016; 44(10):1851±60. doi: 10.1097/CCM. 0000000000001827 PMID: 27355527
7. Gunnerson KJ, Shaw AD, Chawla LS, Bihorac A, Al-Khafaji A, Kashani K, et al. TIMP2·IGFBP7 biomarkerpanel accurately predicts acute kidney injury in high-risk surgical patients. Journal of Traumaand Acute Care Surgery. 2016; 80(2):243±9. doi: 10.1097/TA.0000000000000912 PMID: 26816218
8. Hoste EA, McCullough PA, Kashani K, Chawla LS, Joannidis M, Shaw AD, Feldkamp T, Uettwiller-Geiger DL, McCarthy P, Shi J, Walker MG, Kellum JA, Sapphire Investigators Derivation and validation of cutoffs for clinical use of cell cycle arrest biomarkers. Nephrol Dial Transplant. 2014;29(11):2054–2061. doi: 10.1093/ndt/gfu292
9. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A,Birkhahn R, Cely CM, Chawla LS, Davison DL, Feldkamp T, Forni LG, GongMN, Gunnerson KJ, Haase M, Hackett J, Honore PM, Hoste EA, Joannes-Boyau O, Joannidis M, Kim P, Koyner JL, Laskowitz DT, Lissauer ME, Marx G,McCullough PA, Mullaney S, Ostermann M, Rimmelé T, et al: Discovery andvalidation of cell cycle arrest biomarkers in human acute kidney injury.Crit Care 2013, 17:R25.
10. Kellum JA, Sileanu FE, Murugan R, Lucko N, Shaw AD, Clermont G: Classifying AKI by urine output versus serum creatinine level. JAm Soc Nephrol 26: 2231–2238, 2015
11. Kimmel M, Shi J, Latus J, Wasser C, Kitterer D, Braun N, et al. Association of Renal Stress/Damage and Filtration Biomarkers with Subsequent AKI during Hospitalization among Patients Presenting to the Emergency Department. Clinical journal of the American Society of Nephrology: CJASN. 2016; 11 (6):938±46. Epub 2016/03/31. doi: 10.2215/CJN.10551015 PMID: 27026519
12. Konvalinka A. Urine proteomics for acute kidney injury prognosis: another player and the long road ahead. Kidney Int [Internet].2014 Apr [cited 2015 Jan 13];85(4):735-8.Available from: http: //www .nature. com /ki/ journal /v85/ n4 /pdf/ki2013421a.pdf
13. Meersch M, Schmidt C, Van Aken H, Martens S, Rossaint J, Singbartl K,Görlich D, Kellum JA, Zarbock A: Urinary TIMP-2 and IGFBP7 as earlybiomarkers of acute kidney injury and renal recovery following cardiacsurgery. PLoS One 2014, 9:e93460.
14. Levy JH, Szlam F, Wolberg AS, Winkler A. Clinical Use of the Activated Partial Thromboplastin Time and Prothrombin Time for Screening: A Review of the Literature and Current Guidelines for Testing. Clin Lab Med. 2014 Sep. 34(3):453-477.
15. Li , H et al .(2001)Hum. Gene Ther . 12:515.
16. Malyszko J, Bachorzewska-Gajewska H, Dobrzycki S. Biomarkers of contrast-induced nephropathy. Which ones and what is their clinical relevance? Interventional Cardiology Clinics.2014;3(3):379-91.
17. Murray PT, Mehta RL, ShawA, RoncoC, Endre Z, Kellum JA,ChawlaLS, CruzD ,Ince C, Okusa MD; ADQI 10workgroup: Potential use of biomarkers in acute kidney injury: Report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. Kidney Int 85: 513–521, 2014
18. Pilarczyk K, Edayadiyil-Dudasova M, Wendt D, Demircioglu E, Benedik J, Dohle DS, et al. Urinary[TIMP-2]*[IGFBP7] for early prediction of acute kidney injury after coronary artery bypass surgery.Annals of intensive care. 2015; 5(1):50. Epub 2015/12/17. doi: 10.1186/s13613-015-0076-6 PMID:26669781
19. Remuzzi . G and Horton . R, "Acute renal failure: an unacceptable death sentence globally,"TheLancet, vol. 382, no. 9910, pp. 2041– 2042, 2013.
20. Schantz M , Hoferer A , Shi J , Dominik M , Kimmel M . Urinary TIMP2• IGFBP7 for the prediction of platinum-induced acute renal injury. Int J Nephrol Renovasc Dis. 2017; 10: 175–181.Published online 2017 Jun 28. doi: 10.2147/IJNRD.S135271
21. Tatsumi N, Miwa S, Lewis SM, International Society of Hematology, and the International Council for Standardization in Haematology. Specimen collection, storage, and transmission to the laboratory for hematological tests. Int J Hematol2002; 75:261–8.
22. Vanmassenhove J, Vanholder R, Nagler E, Van BW (2013) Urinary and serum biomarkers for the diagnosis of acute kidney injury: an indepthreview of the literature. Nephrol Dial Transplant 28:254–273
23. Vijayan A , Faubel S , Askenazi DJ , Cerda J , Fissell WH , Heung M , Humphreys BD , Koyner JL , Liu KD , Mour G , Nolin TD ,Bihorac A ; American Society of nephrology , Acute kidney injury advisory group . Am J kidney dis 2016 , 68(1):19-28 .
24. Westhoff JH, Tönshoff B, Waldherr S, Pöschl J, Teufel U, Westhoff TH, Fichtner A.PLoS One. 2015 Nov 25;10(11):e0143628. doi: 10.1371 /journal. pone.0143628. eCollection 2015.PMID:26606754
25. Wetz AJ, Richardt EM, Wand S, Kunze N, Schotola H, Quintel M, et al. Quantification of urinary TIMP-2and IGFBP-7: an adequate diagnostic test to predict acute kidney injury after cardiac surgery? Criticalcare (London, England). 2015; 19:3. Epub 2015/01/07.
26. WHO (2006) WHO Multicentre Growth Reference Study Group. Enrollment and baseline characteristics in the WHO Multicentre Growth Reference Study. Acta Paediatr Suppl. 2006;450:7–15.