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Evaluation of Serum Biomarkers in High Risk Acute Kidney Injury Patients for their Early Detection and Risk Assessment

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Abstract
Background: *Acute kidney injury (AKI) is a leading cause of 5-7% of inpatients wards and 30% of all ICU patients with high morbidity and mortality. The need for early detection of renal injury has recently lead to identification of several biomarkers of AKI, that have been found elevated with clinical AKI, in some cases prior to a gold standard diagnostic threshold(rise in serum creatinine by 50%).*
Objectives: *In search of biomarkers to detect AKI earlier & stratify its risk; cystatin C, Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), IL-6, IL-18 & Uric acid (UA) were tested from patients' serum after satisfying all inclusion & exclusion criterias.*
Materials & Methods: *ELISA (Enzyme Linked Immuno Sorbent Assay) for cystatin C, IL-6, IL-18 and autoanalyzer for LDH, UA, ALP were used to assay them.*
Results: *83.72% of stage 1(Acute Kidney Injury Network system) was found among 61.4% of high risk AKI patients. Maximum rise in creatinine occurs early after 24 hrs, which gradually declines in subsequent days. Level of all biomarkers rise in post operative period from 1.2 to 4 times of baselines. Day 1 UA >4.62mg/dl & >17.36% rise from baseline, rise in cystatin C & IL-18 by 94.23% and 23.4% respectively were set as cut-off to predict AKI. Baseline serum creatinine is the strongest predictor of creatinine at 3 &6 months, while baseline cystatin C has found to be moderate strength of prediction from our study.*
Keywords: *AKI, Cystatin C, LDH, ALP, IL-6, IL-18, UA.*

Introduction

Acute kidney injury (AKI), formarly called acute renal failure (ARF), is defined as an abrupt decline in renal function. Cinically manifesting as a reversible acute increase in blood urea nitrogen (BUN) and serum creatinine levels over the course of hours to weeks. This condition complicates 5-7% of hospital admissions and 30% of all ICU patients with high morbidity and mortality. The diagnosis of AKI is based on either an elevation of serum creatinine and or detection of oliguria, defined by using KDIGO (Kidney Disease Improving Global Outcome) criteria and classified by RIFLE (Risk, Injury, Failure, Loss of kidney

function and End stage kidney disease) or AKIN (Acute Kidney Injury Network) system, which correlate well with severity and outcome. Various studies have assessed the incidence, risk factors and outcome of AKI in patients of cardiac surgery, sepsis, cirrhosis and in ICU settings, however very few have been done in urological patients.

Serum creatinine level is greatly influenced by numerous non renal factors (such as body weight, race, age, gender, total body volume, drugs, muscle metabolism and protein intake). Its utility is worse in early AKI, because the patients are not in steady state; hence, serum creatinine lags far behind renal injury until 48-72 hrs after the initial insult. In addition, significant renal disease can exist with minimal or no change in serum creatinine, because of renal reserve enhancing tubular secretion of creatinine, decreased oral intake of protein, or altered hepatic production. Serum creatinine often does not increase until the GFR has moderately decreased(about 40 ml/min/1.73 m^2). This insensitivity to small to moderate decrease in GFR is the so called creatinine blind GFR area (40-70 ml/min/1.73m^2) may result in an unnecessarily high drug dose thus increasing the further risk to patients. Pre-clinical renal disease has been defined as eGFR >60ml/min/1.73m^2 with normal serum creatinine level and is associated with overt CKD (chronic kidney disease), cardiovascular disease and increase mortality.

The need for early detection of renal injury has recently lead to identification of several biomarkers of AKI, that have been found elevated with clinical AKI, in some cases prior to a gold standard diagnostic threshold (rise in serum creatinine by 50%). These biomarkers include both serum tests such as cystatin C, LDH, ALP, IL-6, IL-18, Uric acid (UA) and urinary testes such as IL-18 and neutrophil gelatinase associated lipocalin (NGAL), along with several others. Uncertainity still exists, however, as to whether these biomarkars possess adequate prognostic accuracy for both established AKI and for early detection of AKI. Limited studies are available that directly compare the performance of these biomarkers as tests for diagnosis of AKI, and their consistency across certain urological subgroups (e.g., obstructive uropathy, urosepsis, post renal surgery, trauma).

Cystatin C, a non glycosylated protein, is produced continuously by all nucleated cells and its level is only determined by GFR. Moreover, its concentration is not influenced much by age, gender, muscle mass, infections, and inflammatory or liver diseases. Several studies demonstrated the superiority of serum cystatin C in comparison with creatinine in the detection of minor GFR reduction. Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) are useful markers that have been found to be raised in critically ill patients with AKI in ICU settings. IL-6 and IL-18 are inflammatory cytokines that has long been described as having both pro and anti-inflammatory properties. These have been found to correlate with onset severity of AKI. Serum uric acid (UA) is the potential marker, which is not only easy to measure and affordable, but also been found to predict the progression of AKI and renal replacement therapy (RRT) requirement particularly in patients underwent cardiac surgeries.

Thus in our study, we will evaluate the accuracy and reliability of six serum biomarkers for the early diagnosis and risk stratification of AKI, in subgroup of patients with normal serum creatinine with possibility of renal function deterioration, consulted under department of urology and biochemistry.

Aims and Objectives

1. To document and analyse the clinical and risk profile of all high risk urological patients (obstructive uropathy, post renal surgery, renal trauma and urinary tract infections).
2. To study incidence of AKI in different subgroup of high risk urological patients.

3. To study the level of serum creatinine, cystatin C, LDH, ALP, IL-6, IL-18 and UA at early stages of AKI.
4. To study prognostic outcome of AKI and its correlation with cystatin C, LDH, ALP, IL-6, IL-18 and UA.

Materials and Methods

Study Area: Dept. of Biochemistry and urosurgery, in our Medical College, Kolkata

Study Population: All patients of obstructive uropathy, UTI, and post renal surgery/ renal trauma with normal serum creatinine level, attending to dept. of urosurgery.

Sample Size: 70 patients.

Sample Design: Urological patients after clinical diagnosis and risk factor profile analysis were divided into three groups: (1) Obstructive uropathy (2) Urinary tract infections (3) Renal surgery/Renal trauma. From these groups, patients were selected for assessing early AKI according to the following criteria –

a. **Inclusion criteria:** high risk group patients ((1) Obstructive uropathy (2) Urinary tract infections (3) Renal surgery/Renal trauma) with serum creatinine upto 1.5 mg/dl and atleast one* predisposing factors:

i) Age above 70 years

ii) Cardiogenic or haemorrhagic shock

iii) Hyperbilirubinemia with serum bilirubin >2 mg%

iv) Chronic heart failure NYHA class III or IV or cardiac surgery or coronary stent placement or bypass

v) Diabetes

vi) Hypertension

vii) Sepsis

viii) Neurological disease or cognitive impairment / disability

ix) Emergency surgery

x) Anaemia

xi) COPD

*Exception– patients undergoing partial or radical nephrectomy and grade IV/V renal trauma were included irrespective of presence of predisposing factors.

b. **Exclusion Criteria**

i) Age <18 years

ii) Elevated serum creatinine >1.5 mg/dl

iii) Hypothyroidism, hyperthyroidism, aortic aneurysm

iv) Patients receiving corticosteroid, trimethoprim, cimetedine, diuretic therapy

Study Design: Prospective observational study

Parameters Studied

a. Age, sex, religion, weight, hight, body surface area, etc of patients.

b. Patients' clinical profile, diagnosis, management and outcome

c. Baseline renal function followed by serial measurement, urin output and eGFR

d. Baseline and repeat serum level of cystatin C, LDH, ALP, IL-6, IL-18 and UA.

e. Incidence and outcome of AKI

f. Follow up measurement of serum creatinine and eGFR for minimum period of 6 months.

Study Technique: All patients after fulfilling inclusion and exclusion criteria were included in this study after proper consent. Detailed documentation and assessment of clinical and biochemical profile of patients were done followed by repeat assay at post operative day 1(24 hrs later) in cases of surgery or post admission day 1 (24 hrs later) in cases of non operative managements. Patients' renal function was strictly followed during the course in hospital. Occurrance of AKI was detected by KDIGO criteria and staged according to AKIN and RIFLE system. Serial monitoring of renal function was done. Final outcome regarding recovery, deterioration of renal function or death was reported. After discharge study patients were followed in OPD for 3 and 6 months. Patients' data was maintained in a chart during this study period. Serum UA, LDH, ALP done by colorimetric methods in autoanalyzer. Cystatin C, IL-6, IL-18 were done by ELISA kit (Ray Biotech, Inc, USA). Serum were stored at -80^{0}C until analysis.

Plan for analysis of data: The collected data was analyzed using SPSS, IBM software, version 21.0. Descriptive analysis was described in mean, standard deviation, range, minimum and maximum value. Comparison between two groups was done with independent sample t test; ANOVA test was used in more than two groups. 0.05 level was taken as statistical significance at 95% confidence interval. Bivariate correlation was used to check association between continuous variables. Chi-square test was utilized for categorical variables. ROC analysis was done to find area under curve (AUC) along with sensitivity and specificity at particular level.

Results and Discussion

Early detection of AKI is of paramount importance for prevention of progression, morbidity and associated mortality of AKI and multi organ dysfunction. Its incidence varies from low (1-5)%, as in routine surgeries to high (30%) in ICU patients. Caddeo G et al, in their study on epidemiology of AKI in urological patients, found an overall incidence of 6.7%. Stage 1 AKI was seen in 61.0% of cases, while 16.1% and 22.6% had stage 2 and 3 AKI respectively[1]. In our study, 61.43% of patients developed AKI (Table 1). Such a high incidence is mainly due to a large selection bias, where patients with high probability of AKI were selected depending on presence of co-morbidities and direct renal surgery or trauma, in order to detect significance of biomarkers. Despite that 83.72% of patients had stage 1 AKI(AKIN criteria) and 13.95% of cases had stage 2 AKI (Table 2). When defined by RIFLE classification, this staging changed slightly with 74.42% of cases in 'R' category and 23.26% of cases in 'I' category. Only one patient has stage 3 or 'F' category AKI (Table 3). All the patients recovered from AKI within 2-7 days period.

Table 1. AKI incidence in study population

		Frequency	Percent
AKI	No	27	38.57
	Yes	43	61.43
	Total	70	100.0

Based on KDIGO guidelines – 2012.

Table 2 Staging of AKI according to AKIN system

AKIN(Acute Kidney Injury Network) System		Frequency	Percent
Stage	1	36	83.72%
	2	6	13.95%
	3	1	2.33%
	Total	43	100

Staging based on serum creatinine and/or urin output

Table 3 Staging of AKI according to RIFLE classification:

Class	Frequency	Percent
R (Risk)	32	74.42%
I (Injury)	10	23.26%
F (Failure)	1	2.32%
L (Loss of kidney function)	0	0
E (End stage kidney disease)	0	0
Total	43	100.0

Subgroup analysis showed more number of young and middle aged participants. 62.9% of participants were male (Table 4). Stone disease was most common diagnosis in nearly 42% of cases, followed by neoplasia in 20% of cases. PCNL, nephrectomy and URSL were the most commonly performed surgeries. Hypertension, diabetes, urosepsis were most common high risk condition associated. 57.1% of patients were in RS group, followed by 28.6% and 14.3% in UTI and OU group respectively (Table 5). Incidence of AKI based on gender, age, diagnostic groups and management categories, revealed similar incidence in all groups.

Table 4. Gender distribution in the study population:

Gender	Frequency	Percent
Male	44	62.9%
Female	26	36.1%
Total	70	100

Sex ratio of male:female = 1.7:1

Table 5. Grouping of the high risk patients in the study

High risk groups	Frequency	Percent
1. Obstructive uropathy (OU)	10	14.3%
2. Renal surgery (RS)	40	57.1%
3. Urinary tract infection (UTI)	20	28.6%
Total	70	100.0

In the studies of AKI in cardiac surgeries' patients, authors reported a rise in serum creatinine after 48-72 hrs of operation[2,3]. Similarly in studies of ICU patients with initial normal creatinine at admission, later rise in creatinine was reported again after 48-72 hrs[4]. However, in our study, rise in serum creatinine in almost all AKI cases, was observed early, after 24 hrs [Post operative day (POD) -1]with a sustained raised levels at day 2 and followed by rapid fall on subsequent days (Table 6). Reason for differing pattern in rise of creatinine may be explained by different pathophysiology of disease in previously stated population. In cardiac surgeries and ICU patients, pre- renal factors are most responsible, however in our series, direct renal insult (renal surgery) and post renal factors (OU group) probably caused a quick rise in creatinine level without any delay.

Table 6. Variation in post operative serum creatinine from baseline and baseline eGFR in study population:

	Baseline eGFR	Baseline S.Cr	Serum Creatinine			
			Day 1	Day 2	Day 3	Day 4
Mean	84.81	0.84	1.41	1.36	1.20	1.00
SD	25.32	0.34	.55	.41	.38	.28

SD – standard deviation. S.Cr – serum creatinine (mg/dl). eGFR – estimated GFR(MDRD equation) in ml/min/1.73m^2.

Study of serum level of biomarkers showed a significant rise in post operative period at day 1 by 1.2 -4 times (Table 7). Rise was seen in cases with or without AKI, however % rise was different. Serum uric acid at day1 was found significantly associated with presence of AKI (P<0.05). Other biomarkers at day 1 were raised, but could not differentiate between AKI v/s non-AKI cases significantly. When percentage rise from baseline in biomarkers were analyzed with AKI presence, it revealed significant association with cystatin C (P<0.05), IL-18(P=0.003) and uric acid (P= 0.006). (Table 8).

Table 7. Serum biomarkers level of study population at baseline and day 1 :

	Baseline	Day1
Cystatin C (mg/L)	1.12±0.18	2.38±0.39
IL – 6 (pg/ml)	1.16±0.68	2.75±0.70
IL – 18 (pg/ml)	173.2±51.34	200.48±32.00
Uric Acid (mg/dl)	3.62±0.72	5.12±0.89
LDH (U/L)	172.72±34.23	408.28±90.24
ALP (U/L)	79.67±27.28	249.0±113.62

All values are given in terms of mean±standard deviation.

Table 8 Percent rise of serum biomarkers from baseline with AKI

	AKI	N	Percent rise (Mean)	Std. Deviation	P value
Cystatin C	Yes	43	117.6%	46.2	**<0.05**
	No	27	80.6%	50.4	
IL – 6	Yes	43	658.3%	1022.4	>0.05
	No	27	610.9%	1203.2	
IL – 18	Yes	43	60.12%	58.1	**<0.05**
	No	27	25.62%	28.3	
UA	Yes	43	36.4%	19.9	**<0.05**
	No	27	20.0%	20.34	
LDH	Yes	43	138.9%	60.49	>0.05
	No	27	127.12%	68.4	
ALP	Yes	43	277.24%	176.7	>0.05
	No	27	223.6%	185.8	

IL- interleukin; UA- uric acid; LDH- lactate dehydrogenase; ALP- alkaline phosphatase

AKI was stratified in risk categories by AKIN staging and RIFLE classification. Though day 1 level of all biomarkers was raised, they failed to show any differences on the basis of AKI risk categories. When renal function was analyzed by eGFR, cystatin C(P=0.04), and uric acid (P=0.05)

at day 1 had mild negative correlation to eGFR change, however a strong negative correlation was found with percentage rise in uric acid (P=0.001) and cystatin C(P=0.000).

On subgroup analysis based on high risk groups, association of day 1 level of uric acid with AKI, was maintained in RS and UTI groups. Possible reason for non significance in OU group may be the limited number of cases in that group. Similarly significance of % change in uric acid and IL-18 were maintained in RS and UTI group, but % change in cystatin C became non significant. So above data suggested a trend towards better association of biomarkers in RS and UTI groups than in OU groups. Fujisawa et al in his series of three cases of post renal AKI found great discrepancy between raised creatinine level and near normal cystatin C level[5]. Similar observations were also reported in an animal model of rats by Tsuda H et al[6]. He found lower level of cystatin C and beta-2-microglobulin in rats with bilateral ureteric obstruction(post renal AKI), in contrast to a much higher level in rats with bilateral nephrectomy (intrinsic AKI). Barbara in his review on biomarkers of AKI mentioned that cystatin C is not diagnostically specific for AKI, it is an marker of glomerular filtration dysfunction than of tubular lesion[7].

ROC analysis in our study revealed that day 1 uric acid >4.62mg% and >17.36mg% rise in uric acid from baseline had 73% sensitivity with 65% specificity and 81% sensitivity and 65% specificity respectively. Similarly >94.23% rise in cystatin C level and >23.4% rise in IL-18 had 76% sensitivity, but a lower specificity (54-67)% (Table 9). Recently Kim JH et al investigated association between uric acid level and mortality and AKI after paraquat intoxication and found significantly higher level of UA in AKI and death groups[8]. Gaipov A et al also reported higher prediction capability of AKI at 24 and 48 hrs with UA and with NGAL, in open heart surgery patients[9]. Apart from an early marker of AKI, UA is now also becoming a potential therapeutic target in kidney diseases[10,11]. Serum IL-6 has been reported has a early marker of AKI in few studies[12,13]. But in these studies, population comprises of children mainly and cases studied had cardiac surgeries. We did not found any association of IL-6 with AKI.

Table 9 Biomarkers cut-off from ROC curve analysis to predict AKI

Biomarkers	Positive if greater than or equal to Cut-off	sensitivity	1-specificity
% rise in uric acid	17.36%	0.83	0.46
Day 1 uric acid (mg/dl)	4.62	0.69	0.27
% rise in cystatin C	94.23%	0.77	0.46
% rise in IL – 18	23.44%	0.77	0.43

Serum and urin IL-18 has shown promising role as biomarker for AKI[14]. Zhu L et al found good correlation of serum IL-18 with sepsis induced AKI in ICU patients[15]. We also found strong association with higher percentage rise of IL-18 with AKI. Serum LDH is a marker of tissue damage and inflammation. Kym D et al in his study on major burn patients found that raised LDH was significantly associated with early AKI and mortality[16]. We found raised LDH level in all post operative patients, irrespective of presence of AKI. ALP is another marker with diagnostic as well as therapeutic potential for sepsis induced AKI[17]. However we did not find it specific for AKI.

Long term renal assessment in our study patients revealed stable function at 3 and 6 months. Previous episode of AKI did not confer any detrimental effect on long run. Reason may be recovery of all patients in immediate post operative period. Prior normal renal function (baseline creatinine <=1.5mg/dl) was the strongest predictor of creatinine level at 3 and 6 months, additionally baseline cystatin C also had good correlation.

Conclusion

Following conclusion were drawn from the study

1) High risk urological patients are on high risk of AKI development in post operative period (61.4%).

2) Most patients of AKI of stage 1(83.72)% and almost all were recovered within 2-7 days.
3) Maximum rise in creatinine occurs early after 24 hrs(POD-1), which gradually comes down in subsequent days, unlike in cardiac surgeries or ICU settings.
4) Level of all biomarkers rise in post operative period from 1.2 to 4 times of baselines.
5) Day 1 level of biomarkers fail to risk stratify the AKI.
6) Day 1 UA >4.62mg% predicts AKI with sensitivity of 73% and specificity of 65%.
7) >17.36%rise in UA from baseline predicts AKI with sensitivity of 81% and specificity of 65%.
8) Rise in cystatin C & IL-18 by 94.23% and 23.4%, predicts AKI with sensitivity of 76% for each and specificity of54% & 67% respectively.
9) Significance of biomarkers appears to fade in obstructive uropathy group, with less rise of biomarkers, despite AKI.
10) Overall long term renal function remains stable at 3 &6 months, in patients recovered from previous AKI.
11) Baseline serum creatinine is the strongest predictor of creatinine at 3 &6 months, while baseline cystatin C has moderate strength of prediction.
12) IL-6, ALP, & LDH are poor predictor of AKI and these are not recommended in routine urological practice.

So, biomarkers have different association strength and predictability power for AKI. Serum uric acid, IL-18 and cystatin C appears to perform better than others. A combination of them and serial measurements may further improve the accuracy.

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References

1. Caddeo G, Williams ST, McIntyre CW et al. Acute kidney injury in urology patients: incidence, causes and outcomes. Nephrourol Mon. 2013 Nov;5(5):955-61.
2. Mehta RL, Chertow GM. Acute renal failure definitions and classification: time for change? J Am Soc Nephrol. 2003 Aug;14(8):2178-87.
3. Bjornsson TD. Use of serum creatinine concentrations to determine renal function. Clin Pharmacokinet. 1979 May-Jun;4(3):200-22.
4. Star RA. Treatment of acute renal failure. Kidney Int. 1998 Dec;54(6):1817-31.
5. Fujisawa N, Yashiro M, Segawa H et al. Discrepancy between cystatin C and creatinine points leading to a diagnosis of post renal acute kidney injury and its reversibility: three case reports. Clin Exp Nephrol. 2010 Dec;14(6):608-13.
6. Tsuda H, Isaka Y, T akahara S et al. Discrepancy between serum levels of low molecular weight proteins in acute kidney injury model rats with bilateral ureteral obstruction and bilateral nephrectomy. Clin Exp Nephrol. 2009 Dec;13(6):567-70.
7. Lisowska-Myjak B. serum and urinary biomarkers of acute kidney injury.Blood Purif . 2010;29(4):357-65.
8. Kim JH, Gil HW, Yang JO et al. Serum uric acid level as a marker for mortality and acute kidney injury in patients with acute paraquat intoxication. Nephrol Dial Transplant. 2011 Jun;26(6):1846-52.
9. Gaipov A, Solak Y, Turkmen K et al. Serum uric acid may predict development of pregressive acute kidney injury after open heart surgery. Ren Fail. 2015 Feb;37(1):96-102.
10. Ejaz AA, Dass B, Lingegowda V et al. Effect of uric acid lowering therapy on the prevention of acute kidney injury in cardiovascular surgery. Int Urol Nephrol. 2013 Apr;45(2):449-58.
11. Canet E, Cheminant M, Zafrani L et al. Plasma uric acid response to rasburicase: early marker for acute kidney injury in

tumour lysis syndrome? Leuk Lymphoma. 2014 Oct;55(10):2362-7.

12. Miklaszewska M, Korohoda P, Zachwieja K et al. Serum interleukin 6 levels as an early marker of acute kidney injury on children after cardiac surgery. Adv Clin Exp Med. 2013 May- Jun;22(3):377-86.
13. Liu KD, Altmann C, Smits G et al. Serum interleukin 6 and interleukin 8 are early biomarkers of acute kidney injury and predict prolonged mechanical ventilation in children undergoing cardiac surgery: a case control study. Crit Care. 2009;13(4):R104.
14. Che M, Xie B, Xue S et al. Clinical usefulness of novel biomarkers for the detection of acute kidney injury following elective cardiac surgery. Nephron Clin Pract. 2010;115(1):c66-72.
15. Zhu L, Shi D. Early diagnostic value of neutrophil gelatinase-associated lipocalin and interleukin 18 in patients with sepsis induced acute kidney injury. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue. 2016 Aug;28(8):718-22.
16. Kym D, Cho YS, Yoon J et al. Evaluation of diagnostic biomarkers for acute kidney injury in major burn patients. Ann Surg Treat Res. 2015 May;88(5):281-288.
17. Pickkers P, Heemskerk S, Schouten J et al. Alkaline phosphatase for treatment of sepsis induced acute kidney injury: a prospective randomized double-blind placebo-controlled trial. Crit Care. 2012 Jan 23;16(1):R14.