RESEARCH

KIDNEY INJURY MOLECULE 1 (KIM 1) AND NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) AS PREDICTIVE DIAGNOSTIC MARKERS OF RENAL DYSFUNCTION IN PATIENTS UNDERGOING MAJOR SURGERY

Received : 13/05/2021
Received in revised form : 19/07/2022
Accepted : 29/07/2022

Keywords:
Acute kidney injury,
KIM1,
NGAL

Corresponding Author:
Dr. Suchitra Chongtham,
Email: sck248@gmail.com
ORCID: 0000-0002-8807-7162

DOI: 10.47009/jamp.2022.4.3.17

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm,
2022; 4 (3); 77-81

Phurailatpam Uma Devi[1], Lamabam Chanchal Chanu[2], Rajkumari Anupama[1], Suchitra Chongtham[3], Rajesh Waikhom[4]

[1]PGT, Department of Biochemistry, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India
[2]Asstistant Professor, Department of Biochemistry, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India
[3]Associate Professor, Department of Biochemistry, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India
[4]Professor, Department of Medicine, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India

Abstract

Background: In patients undergoing surgery, acute kidney injury (AKI) is a common complication. Increase serum Creatinine is the gold standard diagnostic criteria, but certain factors limit its use for early detection. A biomarker that can be used in early and timely detection would represent great advancement in the management of AKI. Several new early biomarkers of kidney dysfunction have been suggested recently, of which Kidney Injury Molecule 1 (KIM1), Neutrophil Gelatinase Associated Lipocalin (NGAL) are the promising ones. The study was taken up to estimate serum levels of KIM1 and NGAL in patients during major surgery and also to determine whether KIM1 and NGAL is a better predictive diagnostic marker of renal dysfunction. **Materials and Methods:** The prospective study was carried out from September 2019 to October 2021 in 50 subjects undergoing major surgery. Using Kidney Disease: Improving Global Outcomes (KDIGO), 2012 criteria an increase in serum creatinine ≥ 26.5µmol/L within 48hours was used to define post-operative AKI. Serum concentration of pre and post-operative KIM1 and NGAL were measured using Sandwich – ELISA method. Statistical analysis was done using SPSS software version 21. **Result:** Out of 50 patients, 6(12%) patients developed post-operative AKI. Older age and diabetes contribute as its risk factors. Post-operative values of KIM1 and NGAL were found to be significantly higher in AKI group as compared to non-AKI. Moderate positive correlation was found between the two biomarkers and creatinine in their post-operative values. ROC-AUC was the highest for NGAL (0.970). **Conclusion:** The study concludes that both the KIM1 and NGAL are raised in post-operative AKI patients. Also, the post-operative concentrations of NGAL and KIM1 have high sensitivity and specificity and both the biomarkers also has moderate positive correlation with serum Creatinine. Thus, serum KIM1 and serum NGAL could be a promising biomarker in the diagnosis of post-operative AKI.

INTRODUCTION

Acute kidney injury (AKI) is recognised as one of the serious post-operative complications. Using Kidney Disease: Improving Global Outcomes (KDIGO) serum creatinine (serum Cr) criteria, the incidence of post-operative AKI varies from around 1% after general surgery to as high as around 30% after cardiac surgery.[1,2]

Serum Cr is used as gold standard diagnostic criterion for AKI; but its use is limited due to its inability to indicate initial stage of renal injury,[3] and also its value is influenced by muscle mass.[4] It is also detected after 48hrs of ischemic insult. Thus, a biomarker, like troponin in case of myocardial injury, that can be used in early detection would represent a great advancement in the management of AKI.

Kidney Injury Molecule (KIM1) is a type 1 trans-membrane glycoprotein. It is primarily expressed on the surface of T- cells and has two extracellular domains. Its expression is low in normal kidneys, but markedly increased succeeding kidney injury in proximal tubular cells.[5] The reason being ectodomain of KIM1 undergoes cleavage and can be detected in blood and urine.[6] Neutrophil Gelatinase Associated Lipocalin (NGAL) is an acute-phase glycoprotein secreted in minute amounts by neutrophils, epithelial cells of various organs.[7] Its expression is reported to be significantly increased when it responds to cellular stress and is highly induced protein in the kidney after ischemic insult.[8] It is present both is plasma and urine.[9]

The present study is taken up to estimate serum levels of KIM1 and NGAL in patients during major operative surgery and to determine whether KIM1 and NGAL is a better predictive diagnostic marker of renal dysfunction.

MATERIALS AND METHODS

This was a prospective hospital based study carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Department of Surgery, Jawaharlal Nehru Institute of Medical Sciences, Porompat, Manipur from September 2019 to October 2021. After taking informed consent, a total of 50 subjects undergoing major surgery, aged 18 years and above were included in the study. Patients with history of end stage renal disease, chronic kidney disease, history of nephrectomy or kidney transplant, patient undergoing dialysis, severely ill patients, or person who is at reactive phase of autoimmune disease were excluded from the study. Using KDIGO, 2012 criteria,[10] an increase in serum creatinine ≥ 26.5µmol/L within 48hours was the criteria to define AKI.

Blood samples were collected before the operation and just at the end of the operation to assess the two biomarkers- KIM1 and NGAL. After separation of serum from the sample, estimation of level of biomarkers was done on the same day using Sandwich – ELISA (Enzyme-linked Immunosorbent Assay) using Elabscience® Human (NGAL) Neutrophil Gelatinase Associated Lipocalin ELISA kit and Elabscience® Human KIM-1(Kidney Injury Molecule 1) ELISA Kit. Again 2ml of blood sample was collected after 48hrs of post-operative period for estimation of serum creatinine and was estimated using enzymatic creatinine amidohydrolase method.

Statistical analysis was done using SPSS software version 21. P-value of <0.05 was considered as statistically significant. The quantitative variables were expressed as mean ± standard deviation (SD). The correlations between the biomarkers were calculated using Pearson's correlation. Areas under the ROC curve were calculated for the serum concentration of post-operative biomarkers. The study was approved by Institutional Ethics Committee, JNIMS, Imphal.

RESULTS

In this study, a total of 50 patients undergoing major operative surgery were included. The mean age of patients (n=50) was (43.42±15.22) years [Table 1].

Table 1: Baseline characteristics of the patients.

Characteristics	Values
Age (in years) [mean±SD]	43.42 ± 15.22
Male (n,%)	32(64%)
Female(n%)	18(36%)
BMI (kg/m2) [mean±SD]	22.65±2.65
Hypertension(n,%)	9(18%)
Diabetes mellitus(n,%)	8(16%)
Carcinoma(n,%)	9(18%)
Emergency surgery (n,%)	28(56%)
Elective operative(n,%)	22(44%)
Serum creatinine(µmol/L) [mean±SD]	71.6±18.6
Serum KIM1(pg/mL) [mean±SD]	113.92±66.35
Serum NGAL(ng/mL) [mean±SD]	42.94±15.85

A total of 6 patients (12%) developed post-operative-AKI. In this study, older age group and diabetes contributes in the risk factor of development of AKI. The post-operative AKI patients have higher mean value of serum KIM1 in both pre-operative and post-operative conditions than non-AKI patients [Table 2]. For those patients who developed post-operative AKI, the post-operative values of serum KIM1 and serum NGAL are increased to almost double their baseline values in their pre-operative period, but these changes are insignificant [Table 3]. All the three biomarkers were found to have positive moderate significant correlation [Table 4].

The AUC for serum NGAL at the cut off 68ng/mL was 0.97 with 100% sensitivity and 88.6% specificity. The AUC for serum KIM1 is 0.888 at a cut off value 119.50pg/mL with 100% sensitivity and 63.6% specificity. Sensitivity of serum creatinine, serum KIM1 and serum NGAL are same. Specificity of serum NGAL (88.6%) was highest followed by serum Cr (72.7%) and serum KIM1 (63.3%) [Table 5].

Table 2: Post-operative characteristics of the patients.

Characteristics	Patient with AKI	Patient without AKI	p- Value
Age (years)	62.33±5.32	40±14.28	0.0007
Gender (n, %) Male (n,%) Female(n,%)	6(12%) 3(9.4%) 3(16.7%)	44(88%) 29(90.6%) 15(83.3%)	0.654
BMI (kg/m2)	21.97±2.09	22.97±1.75	0.2096

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Hypertension(n,%)	3(33%)	6(66%)	0.063
Diabetes mellitus(n,%)	3(37.5%)	5(62.5%)	0.044
Carcinoma(n,%)	3(33.3%)	6(66.7%)	0.063
Emergency surgery (n,%)	3(10.7%)	25(89.3%)	1.000
Elective surgery (n,%)	3(13.6%)	19(86.4%)	
Serum Cr (µmol/L) (in mean±SD) Pre-operative Post-operative	76.02±29.17 115.80±23.87	70.72±16.79 75.14±22.98	0.5257 0.0003
Serum KIM1 (pg/mL) (in mean±SD) Pre-operative Post-operative	169.66±57.93 226.33±59.87	101.93±60.05 112.25±64.16	0.0123 0.0002
Serum NGAL (ng/mL) (in mean±SD) Pre-operative Post-operative	51.66±11.84 90.5±9.97	41.75±16.06 46.68±17.50	0.1526 <0.0001

Table 3: Comparison between pre-operative and post-operative values of the biomarkers in patients who developed AKI.

Biomarkers	Pre-operative (in mean± SD)	Post-operative (in mean±SD)	Correlation	p-value
Serum KIM1 (pg/mL)	169.66±57.93	226.33±59.87	0.777	0.069
Serum NGAL (ng/mL)	51.66±11.84	90.5±9.97	0.711	0.113

Table 4: Correlation between post-operative biomarkers.

Post-operative biomarkers	Correlation (PEARSON)	p- value
Post-op sCr (µmol/L) & Post-op sKIM 1 (pg/mL)	.406	.003
Post-op sCr (µmol/L) & Post-op sNGAL (ng/mL)	.576	.000
Post-op sKIM 1 (pg/mL) & Post-op sNGAL (ng/mL)	.536	.000

Table 5: Area under the curve of the biomarkers

Biomarkers	Cut-off	Sensitivity	Specificity	AUC
Post-operative serum creatinine	83.98 µmol/L	100%	72.7%	0.867
Post-operative serum KIM 1	119.50 pg/mL	100%	63.6%	0.888
Post-operative serum NGAL	68 ng/mL	100%	88.6%	0.970

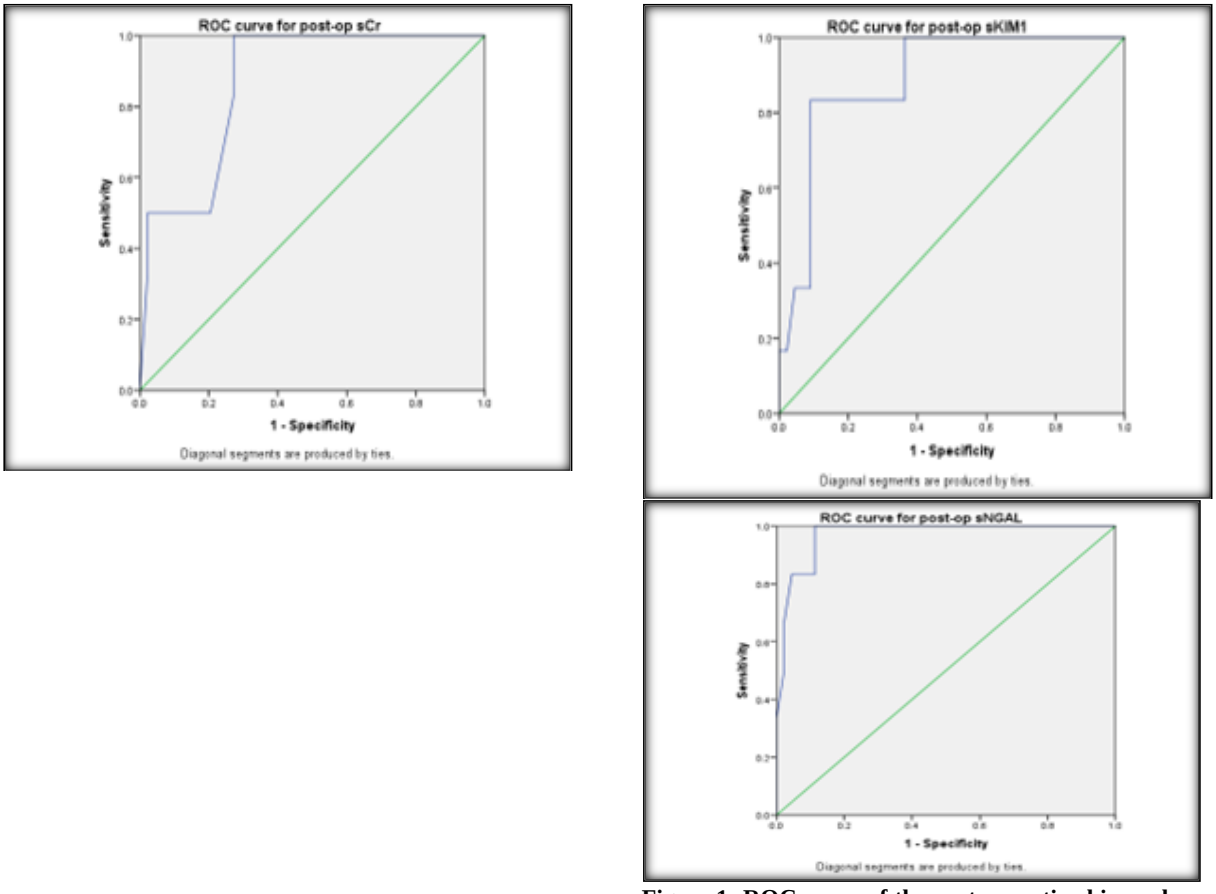

Figure 1: ROC curve of the post-operative biomarkers i) serum creatinine ii) serum KIM1, iii) serum NGAL.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

DISCUSSION

Although serum Cr has a role in predicting post-operative AKI, but it is measured 48 hours after the operation. In this study, post-operative serum NGAL has a statistical significant ($p<0.0001$) value of 90.5 ± 9.97ng/mL in AKI group and 46.68 ± 17.50ng/mL in non-AKI group. The mean post-operative serum NGAL in the AKI was nearly double the value of non-AKI. Similar result was found in a meta-analysis by Hasse et al,[11] in which NGAL was reported to be highly successful in the detection of post-operative AKI. In a study on 1219 patients undergoing open-heart surgery, Koyner et al,[12] reported that plasma NGAL level were more successful than urinary NGAL in detecting AKI and the cut off values for plasma NGAL level was determined to be 323 mg/dL. The different NGAL values may be due to the variation in the time of sampling in the post-operative period, the measurement technique applied or the type of operation.

In this study, the mean value of serum KIM1 in post-operative AKI patients were significantly higher than non-AKI in both pre-operative and post-operative values. It is in concordance with a study by Zdziechowska M et al,[13] in which they reported a 24 hours post-operative serum KIM1 values of 232.6 pg/mL in AKI and 103.7 pg/mL in non-AKI patients. Tubular damage is the most common cause of AKI occurring after surgery.[14] The fact that serum KIM1 is not expressed in the normal kidney and it is up-regulated at the time of injury and inserted into the apical membrane of proximal tubule may be the reason for its increased in the post-operative AKI patients.[15]

For those patients who developed post-operative AKI, the post-operative values of serum KIM1 and serum NGAL are increased to almost double their baseline values in their pre-operative period, but these changes are found to be statistically insignificant. The AUC for serum NGAL at the cut-off 68 ng/mL was 0.97 with 100% sensitivity and 88.6% specificity. Contrary to this, Introcaso G et al.[16] found a lesser area under the curve of 0.71 with 76% sensitivity and 59% specificity at cut-off 154 ng/ml. The difference may be due to variations in time of sample collection and setting cut off at a higher value. The AUC for serum KIM1 is 0.888 at a cut off value 119.50 pg/mL with 100% sensitivity and 63.6% specificity.

Sensitivity of serum creatinine, serum KIM1 and serum NGAL are same. Specificity of serum NGAL (88.6%) was highest followed by serum Cr (72.7%) and serum KIM1 (63.3%). All the three biomarkers were found to have moderately significant positive correlation. This implies that information that we get from serum Cr measured at 48hrs after surgery is obtained from serum KIM1 and serum NGAL as early as just at the end of operation

CONCLUSION

Post-operative concentrations of serum KIM1 and serum NGAL were found to be significantly higher in the patients who developed post-operative AKI as compared to those who do not develop AKI. Post-operative concentrations of serum KIM1and serum NGAL both have high sensitivity and specificity. Both the biomarkers also have moderate positive correlation with serum Cr. So, considering the time lag of almost 48 hours between the assessment of serum Cr and the two biomarkers – serum KIM1 and serum NGAL, the two biomarkers could represent serum Cr in the diagnosis of AKI.

The study concludes that serum KIM1 and serum NGAL are raised in post-operative AKI patients and could be a promising biomarker in the diagnosis of post-operative AKI. However, limitations are faced owing to the small sample size and assessment of biomarkers only once in the post-operative period. So, a well-designed study, with a larger sample size, with serial assessment of the biomarkers – serum KIM1 and serum NGAL, in the post-operative period is suggested to help early detection of AKI in cases where post-operative AKI is anticipated to enable active intervention during the golden hour of AKI, well ahead of detection by the serum Cr level.

Acknowledgment:

It is indeed a privilege to be able to appreciate the Department of Biochemistry, Department of Surgery, Department of Medicine of JNIMS, Imphal for providing all the facilities to carry out this work.

REFERENCES

1. Kheterpal S, Tremper KK, Heung M, Rosenberg AL, Englesbe M, Shanks AM, et al. Development and validation of an acute kidney injury risk index for patients undergoing general surgery: results from a national data set. Anesthesiology. 2009;11:505-15.
2. Rosner MH, Okusa MD. Acute kidney injury associated with cardiac surgery. Clin J Am Soc Nephrol. 2006;1(1):19-32. doi: 10.2215/CJN.00240605.
3. Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. J Am Soc Nephrol. 2011;22(5):810-20.
4. Uwaezuoke SN, Ayuk AC, Muoneke VU, Mbanefo NR. Chronic kidney disease in children: Using novel biomarkers as predictors of disease. Saudi J Kidney Dis Transpl. 2018;29(4):775-84.
5. Jin Y, Shao X, Sun B, Miao C, Li Z, Shi Y. Urinary kidney injury molecule 1 as an early diagnostic biomarker of obstructive acute kidney injury and development of a rapid detection method. Mol Med Rep. 2017;15(3):1229-35.
6. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A,et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol. 2014;25(10):2177-86. doi: 10.1681/ASN.2013070758.
7. Bauvois B, Susin SA. Revisiting Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Cancer: Saint or Sinner? Cancers (Basel). 2018;10(9):336.
8. Tsigou E, Psallida V, Demponeras C, Boutzouka E, Baltopoulos G. Role of new biomarkers: functional and structural damage. Crit Care Res Pract. 2013;2013:361078. doi: 10.1155/2013/361078.
9. Magalhães P, Pontillo C, Pejchinovski M, Siwy J, Krochmal M, Makridakis M, et al. Comparison of Urine and Plasma Peptidome Indicates Selectivity in Renal Peptide Handling.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Proteomics Clin Appl. 2018;12(5):e1700163. doi: 10.1002/prca.201700163.
10. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract. 2012;120(4):c179-84. doi: 10.1159/000339789.
11. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009;54(6):1012-24. doi: 10.1053/j.ajkd.2009.07.020.
12. Koyner JL, Garg AX, Coca SG, Sint K, Thiessen-Philbrook H, Patel UD, et al. Biomarkers predict progression of acute kidney injury after cardiac surgery. J Am Soc Nephrol. 2012;23(5):905-14.
13. Zdziechowska M, Gluba-Brzózka A, Poliwczak AR, Franczyk B, Kidawa M, Zielinska M, et al. Serum NGAL, KIM-1, IL-18, L-FABP: new biomarkers in the diagnostics of acute kidney injury (AKI) following invasive cardiology procedures. Int Urol Nephrol 2020;52(11):2135-43.
14. Park JT. Postoperative acute kidney injury. Korean J Anesthesiol. 2017;70(3):258-266. doi: 10.4097/kjae.2017.70.3.258.
15. Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. Nephrol Dial Transplant. 2009;24(11):3265-8. doi: 10.1093/ndt/gfp010.
16. Introcaso G, Nafi M, Bonomi A, L'Acqua C, Salvi L, Ceriani R, et al. Improvement of neutrophil gelatinase-associated lipocalin sensitivity and specificity by two plasma measurements in predicting acute kidney injury after cardiac surgery. Biochem Med (Zagreb). 2018;28(3):030701.

ISSN (O): 2687-5365; ISSN (P): 2753-6556