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KEY WORDS: Kidney Injury Molecule-1, Acute Kidney Injury, Sepsis, Serum Creatinine, Blood urea nitrogen.

URINE KIDNEY INJURY MOLECULE-1 AS A RAPID DIAGNOSTIC TOOL FOR SEPTIC ACUTE KIDNEY INJURY

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ABSTRACT

Background Acute Kidney Injury (AKI) is an abrupt decrease in kidney function within hours, which includes structural injury as well as impairment of function. Human Kidney Injury Molecule-1 is a type1 membrane-spanning glycoprotein whose expression on the kidney tubular epithelial cells imparts them the capacity to phagocytose dead cells following ischemic injury. **Aims:** To estimate urine KIM-1 level in cases of individuals with septic AKI , to compare urine KIM-1 level with serum Creatinine (SCr) and blood urea nitrogen(BUN), and to assess the usefulness of urine KIM-1 as an early tool to detect AKI. **Statistical analysis** Student's t-test was applied to the data and p-value<0.05 was taken as significant. Pearson's correlation coefficient was used to assess correlation between measured parameters. **Materials and methods** This was a case-control study, in which a total of 100 patients admitted for sepsis at Government Thanjavur Medical College and hospital, Thanjavur, were enrolled from February 2014 to September 2014. Patients diagnosed with AKI were taken as cases and those who did not advance to AKI were considered controls. Samples were collected and estimated for urine KIM-1, serum creatinine and blood urea nitrogen. **Results :** A significant rise in urine KIM-1 was noticed among cases of septic AKI. The study revealed that urine KIM-1 levels significantly rose within 24 hours of admission, whereas, SCr and BUN values were not increased until the third day of nephrotoxic insult. There was no correlation found between levels of urine KIM-1 and, SCr and BUN on the date of admission. With progressive damage to the kidneys, an increase in SCr and BUN levels showed a positive correlation with KIM-1 on third day. **Conclusion:** As an early indicator of renal injury, urine Kidney Injury Molecule-1 shows promise in place of conventional biomarkers of acute kidney injury as SCr and BUN.

INTRODUCTION

Acute kidney injury is characterized by an abrupt decline in the GFR over hours to days. The severity of AKI varies from mild elevations of serum creatinine (SCr) to complete renal failure [1].It is now recognized that AKI may increase the risk of developing chronic kidney disease (CKD), end-stage renal disease, and mortality for years to come [2]. The most common cause of in-hospital mortality has been found to be sepsis[3]. In sepsis, the pathogenesis of AKI includes hemodynamic variations , dysfunction of endothelium, activation of coagulation cascade, renal parenchymal infiltration by inflammatory cells, and tubular obstruction with necrotic cellular debris [4]. Oxidative stress also escalates in sepsis. Multimers of von Willebrand factor (vWF) is secreted from endothelium by the action of reactive oxygen species (ROS) , inducing microvascular thrombosis, ischemia and failure [5]. Further, the necrotic renal tubular cells release ATP into the extracellular space, which through P2X7 receptor directly activates Nrlp3 inflammosome, further worsening the damage [6].

Kidney injury molecule-1 (KIM-1) is a proximal renal tubular membrane spanning glycoprotein which gets markedly expressed in acute nephrotoxic kidney injury[7]. KIM-1 belongs to superfamily of immunoglobulin gene (IgSF), and is structurally close to 'mucosal addressin cell adhesion molecule-1' (MAdCAM-1) [8]. It is a 104kDa type-1 membrane glycoprotein having an extracellular domain with close similarity to immunoglobulin with distinctive six-cysteine residues, a mucin domain capable of getting O-glycosylated and two recognized sites of N-glycosylation [9]. Furthermore, it contains a domain that spans the membrane, and an intracellular domain with a site that can get phosphorylated by tyrosine kinase, proposing that KIM-1 may function as a signalling molecule [7,10]. Gene of KIM-1 is not expressed in normal kidney[7]. Following a nephrotoxic or ischemic insult, KIM-1 gets dramatically upregulated in the proximal convoluted tubules, particularly in the apical membrane of dedifferentiated epithelium[10]. In consequence, the highly glycosylated extracellular part of KIM-1 undergoes rapid proteolytic cleavage by matrix metalloproteinases and is cast off from the apical surface into lumen of the tubules[9]. This delivers a soluble 90kDa part of KIM-1 into the lumen[11]. According to a study done by Vishal S. Vaidya et al., the KIM-1 ectodomain shed in urine can be adequately stable for an extended period of time [9,12]. These findings show that KIM-1expression in renal tissue closely correlates with its release in urine, hence enhancing its utility as a biomarker of AKI [13].The traditional markers, serum creatinine(SCr) and blood urea nitrogen(BUN) have been proved insensisitive as well as non-specific particularly in case of AKI[9].Therefore, this study was done for estimating urine KIM-1 levels in patients with sepsis-induced AKI and also to compare the levels of urine KIM-1 with traditional markers of AKI, SCr and BUN so as to assess the utility of urine KIM-1 as an early predictive marker of sepsis-induced AKI.

MATERIALS AND METHODS

A case-control study was conducted from February 2014 to September 2014 at Government Medical College, Thanjavur. 100 patients admitted in ICU with sepsis were recruited and an informed consent was obtained from each of them. According to the minimum sample required for the study and analysing the feasibility of patient availability during the study period of 8 months in accordance with inclusion criteria, study sample was chosen by convenient sampling method.

Inclusion criteria : Males and females over the age of 18 years were included. The criteria for clinically diagnosing sepsis required an infectious focus along with any two signs of systemic inflammatory response syndrome that included body temperature <36°C or >38°C, pulse rate >90/minute, respiratory rate >20/min and abnormal white blood cell count (>12×103/mm3, <4×103/mm3 or >10% bands) [14].

Exclusion criteria: patients with elevated SCr on the day of admission, patients with pre-existing chronic kidney disease, autoimmune disease, history of Diabetes Mellitus, known hypertensive patients on treatment, history of nephrotic syndrome and Polycystic Kidney Disease were excluded from the study.

AKI was defined as rise in SCr levels of more than 1.5 times from the baseline. Correspondingly the study population was split into two groups. Those in whom AKI was diagnosed

within 12 hours were included as cases and those without AKI as controls. The procedures and methods performed were in agreement with the standards of institutional ethical committee(IECC approval no.007 dated 06.12.2013, Thanjavur Medical college, TN Dr.MGR Medical University).

Specimen collection :From each patient, urine sample was collected on day1 and serum sample on day 1 and day 3. Both serum and urine samples were centrifuged at 2000 rpmfor 20 minutes. The supernatants were stored in aliquots at - 20°C.

KIM-1 estimation by ELISA method:

Urine human KIM-1 was estimated by KIM-1 (human) Enzyme-Linked Immune Sorbent Assay (ELISA)kit(from Hysel India Pvt. Ltd., New Delhi; assay design : M/s Enzo Life Sciences, Switzerland; catalogue no. ADI-900-226-0001). It was based on the principle of double antibody sandwich ELISA. Values below 1ng/ml were regarded as normal.

The serum samples were quantitatively estimated for SCr spectrophotometrically, using chemical kits based on Modified Jaffe's reaction, initial rate method and BUN by Urease-Glutamate Dehydrogenase (GLDH) fixed time method, in fully automated biochemical analyser(EM 360, Transasia).

STATISTICAL ANALYSIS

All data were presented as mean±standard deviation. Differences between group means were statistically analysed by Student's t test. p-Value < 0.05 was considered statistically significant. Pearson's correlation coefficient was used to test the correlation between measured parameters.

RESULTS

Out of 100 septic patients who were enrolled for the study, 49 patients were diagnosed to have AKI. *p-value>0.05 was regarded as statistically insignificant; **p-value≤0.05 as statistically significant and ***p-value≤0.001 as highly statistically significant.

Table/Fig-1: Urine KIM-1 compared among cases and controls.

Urine KIM-1 in	Range (ng/ml)	Mean ± Std. Dev. (ng/ml)	p-value (t-test)
Patients with no AKI (controls)	0.3 - 0.9	0.54± 0.20	<0.001 **
Patients with AKI (cases)	1.3 - 7.8	4.51± 2.25	

Table/Fig-2:SCr compared among the study groups on 1st and 3rd day of hospital admission

Scr values on	Controls	Cases	p-value (t-test done)
	Mean ± SD	Mean ± SD	
Day 1	0.86± 0.18	1.06± 0.50	0.054*
Day 3	1.02± 0.17	3.66± 2.6	<0.001**

Table /Fig-3: BUN compared among the study groups on 1st and 3rd day of hospital admission

BUN values on	Controls	Cases	p-value (t-test)
	Mean ± SD	Mean ± SD	
Day 1	19.3 ±.1.4	23.43±1.7	0.11*
Day 3	20.43±.1.8	48.7±8.1	<0.001**

Table/Fig- 4: Correlation between urine KIM-1,SCr and urine KIM-1, BUN on day 1 and day 3.

Parameters correlated	Correlation coefficient	Correlation	p-value
KIM-1 and SCr on day 1	0.253	Negligible correlation	0.22*
KIM-1 and SCr on day 3	0.888	High positive correlation	<0.001***
KIM-1 and BUN on day 1	0.429	Low positive correlation	0.03**
KIM-1 and BUN on day 3	0.871	High positive correlation	<0.001***

DISCUSSION

SCr has been used till date as a routine standard test to detect AKI. Nevertheless, SCr is not suitable to detect AKI at the earliest because, hike in SCr is observed only after at least 50% renal cells have died and moreover, it has poor sensitivity to slight changes in GFR. As a consequence, it urges the need for better and more sensitive AKI markers. The usefulness of the novel marker urine KIM-1 has been evaluated in our study for speedy diagnosis of AKI in contrast to the regular markers, BUN and SCr.

Patients with septic AKI were found to have urine KIM-1 levels significantly greater (p<0.001) than in those without AKI on the day of ICU admission, as explained in a study by Yuexing Tu et al.,[14] Analyzing SCr and BUN in cases and controls, and comparing their values on day one and day three of admission, revealed no significant difference on admission day, but a highly significant difference on third day. According to our findings, nephrotoxic insult results in an increase in BUN and SCr only on day 3, although urinary KIM-1 concentration rises quickly before conventional biomarker levels do (SCr and BUN). The research of Vishal S. Vaidya et al.,validated these conclusions.[12].

Additionally, on the day of admission it was shown that there was a low correlation between urine KIM-1 and SCr (r = 0.253, p = 0.22); and, there was a weakly positive correlation between urine KIM-1 and BUN (r = 0.429, p = 0.03).On day 3, there was a substantially high positive correlation between KIM-1 levels and SCr (r value 0.888, p 0.001) as well as BUN (r value 0.871, p 0.001), indicating that the kidneys were gradually becoming damaged. As a result, urinary KIM-1 is raised in the early stages of AKI, which is consistent with research by Yuzhao Zhou et al. [15].

According to the aforementioned findings, urinary KIM-1, as opposed to more traditional markers like blood urea nitrogen and serum creatinine, is a specific and non-invasive method for the quick detection of AKI, urine KIM-1 responds substantially to even a small impairment in kidney function [16]. .Urinary KIM-1 is a highly useful biomarker for detecting kidney damage within a day in the event of acute ischemic tubular necrosis [17,18].

The proximal tubules' epithelial cells get dedifferentiated and greatly increase KIM-1 expession in response to acute tubular injury. By means of matrix metalloproteinases that are activated by mitogen activated protein kinase (MAPK) pathway, the extensively glycosylated outer domain of KIM-1 is discharged from the cells' surface into the tubules' lumen, where it is converted into a soluble 90kDa molecule that is excreted in the urine[11].

Importantly, the unique qualities of KIM-1 that make it desirable as a diagnostic marker include the fact that are that it is almost completely missing in healthy kidneys and that its translation is restricted to the cells of afflicted proximal tubules of the kidney. An extracellular domain is promptly split and is noticeable in urine in less than 12 hours when compared to BUN and SCr[19]. KIM-1 could therefore be used as a quick, non-invasive, accurate and reproducible urinary marker to evaluate renal damage[20,21]. Owing to the excellent stability of KIM-1 in urine in spite of multiple freezing and thawing, no stabilizing buffer is needed while storing the samples to prevent its degradation[14,22]. As KIM-1 expresses before proximal tubular epithelial cells are fatally damaged, kidney injury can be treated and reversed quickly [10]. KIM-1 determined by ELISA is linked with low interference from other urinary components and is unaltered

by any physical or chemical modifications in urine[12].

CONCLUSION

Compared to conventional biomarkers like serum urea and serum creatinine, urine KIM-1 was revealed to be a promising fast predictor of acute kidney injury (AKI) in this investigation on patients with septic acute renal injury.

This unique biomarker makes it easier to diagnose AKI early and to make treatment decisions, such as implementing appropriate preventive and therapeutic plans, hence lowering the morbidity and mortality rates related to AKI.

REFERENCES

[1] Wang, H. E., Muntner, P., Chertow, G. M., & Warnock, D. G. (2012). Acute kidney injury and mortality in hospitalized patients. American Journal of Nephrology, 35(4), 349–355.

[2] Humphreys, B. D., Xu, F., Sabbisetti, V., Grgic, I., Movahedi Naini, S., Wang, N., Chen, G., Xiao, S., Patel, D., Henderson, J. M., Ichimura, T., Mou, S., Soeung, S., McMahon, A. P., Kuchroo, V. K., & Bonventre, J. V. (2013). Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. Journal of Clinical Investigation, 123(9), 4023–4035.

[3] Eswarappa, M., Gireesh, M. S., Ravi, V., Kumar, D., & Dev, G. (2014). Spectrum of acute kidney injury in critically ill patients: A single centre study from south India. Indian Journal of Nephrology, 24(5), 280–285.

[4] Zarjou, A., & Agarwal, A. (2011). Sepsis and acute kidney injury. Journal of the American Society of Nephrology, 22(6), 999–1006.

[5] Montini, L., De Pascale, G., M., Pennisi, A., Tanzarella, E. S., & Cutuli, S. L. (2013). A Occhionero, R De Christofaro, M Antonelli. Platelet associated oxidative stress and ADAMTS-13 levels are inversely associated with a poor prognosis in septic shock. Critical Care, 17(supply 2), 13.

[6] Pickkers, P., Heemskerk, S., Schouten, J., Laterre, P.-F., Vincent, J.-L., Beishuizen, A., Jorens, P. G., Spapen, H., Bulitta, M., Peters, W. H. M., & van der Hoeven, J. G. (2012). Alkaline phosphatase for treatment of sepsis-induced acute kidney injury: A prospective randomized double-blind placebo-controlled trial. Critical Care, 16(1), R14.

[7] Huo, Wenqian, Zhang, K., Nie, Z., Li, Q., & Jin, Fengshuo. (2010). Kidney injury molecule-1 (KIM-1): A novel kidney- specific injury molecule playing potential double- edged functions in kidney injury. Transplantation Reviews, 24(3), 143–146.

[8] Ichimura, T., Bonventre, J. V., Bailly, V., Wei, H., Hession, C. A., Cate, R. L., & Sanicola, M. (1998). Kidney Injury Molecule-1 (KIM-1), a putative cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. Journal of BiologyChem, 273, 4135–4142.

[9] Joseph, V. (2009). Bonventer . Kidney injury molecule-1 (KIM-1) : a urinary biomarker and much more. Nephrology Dialysis Transplantation, 24, 3265–3268.

[10] Pennemans, V., De Winter, L. M., Munters, E., Nawrot, T. S., Van Kerkhove, E., Rigo, J. M., Reynders, C., Dewitte, H., Carleer, R., Penders, J., & Swennen, Q. (2011). The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure: A pilot study. Environmental Health: A Global Access Science Source, 10, 77.

[11] Bailly, V., Zhang, Z., Meier, W., Cate, R., Sanicola, M., & Bonventre, J. V. (2002). Shedding of Kidney Injury Molecule-1, a putative adhesion protein involved in renal regeneration. Journal of Biological Chemistry, 277(42), 39739–39748.

[12] Vaidya, V. S., Ramirez, V., & Takaharu Ichimura, N. A. (2006). Bobadilla and Joseph V. Bonventre. Urinary Kidney injury molecule-1: A sensitive quantitative biomarker for early detection of kidney tubular injury. American Journal of Physiology. Renal Physiology, 290:F 517-F 529.

[13] Rees, A. J., & Kain, R. (2008). KIM-1/Tim-1: From biomarker to therapeutic target? Nephrology, Dialysis, Transplantation, 23(11), 3394–3396.

[14] Tu, Y., Wang, H., Sun, R., Ni, Y., & Ma, L. Fei Xv, Xiuping Hu, Lingzhi Jiang, Aiping Wu, Xv Chen, Minhua Chen, Jingquan Liu, and Fang Han. Urinary netrin-1 and KIM-1 as early biomarkers for septic acute kidney injury. Ren Fail. 2014; 36(10):1559–1563.

[15] Zhou, Yuzhao, Vaidya, V. S., Brown, R. P., Zhang, J., Rosenzweig, B. A., Thompson, K. L., Miller, T. J., Bonventre, J. V., & Goering, P. L. (2008). Comparison of kidney injury Molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury, and chromium. Toxicological Sciences, 101(1), 159–170.

[16] Ahmed, S. A., & Hamed, M. A. (2015). Kidney injury molecule-1 as a predicting factor for inflamed kidney, diabetic and diabetic nephropathy Egyptian patients. Journal of Diabetes and Metabolic Disorders, 14, 6.

[17] Shao, X., Tian, L., Xu, W., Zhang, Z., Wang, C., Qi, C., Ni, Z., & Mou, S.. (2014). Diagnostic value of urinary kidney injury molecule 1 for Acute Kidney Injury: A Meta-Analysis. PLOS ONE, 9(1), e84131.

[18] Han, W. K., Bailly, V., Abichandani, R., Thadhani, R., & Bonventre, J. V. (2002). Kidney Injury Molecule-1 (KIM-1): A novel biomarker for human renal proximal tubule injury. Kidney International, 62(1), 237–244.

[19] Vermeulen, R., Zhang, L., AnnejetSpierenburg, X. T., Bonventre, J. V., Reiss, B., Shen, M., Smith, M. T., ChuangyiQiu, Y. G., Ji, Z., Xiong, J., He, J., Hao, Z., Songwang Liu, Y.X., Fei, Y., Weihong, G., Mark Purdue, L. E., Beane Freeman, V. S., Li, Laiyu, Hanlin, H., . . . Qing, L. (2012). Elevated urinary levels of kidney injury molecule-1 among Chinese factory workers exposed to trichloroethylene. Oxford University Press Publications, 1–4.

[20] Szeto, C. C., Kwan, B. C., Lai, K. B., Lai, F. M., Chow, K. M., Wang, G., Luk, C. C., & Li, P. K. (2010). Urinary expression of kidney injury markers in renal transplant recipients. Clinical Journal of the American Society of Nephrology: CJASN, 5(12), 2329–2337.

[21] Malyszko, J., Koc-Zorawska, E., Malyszko, J. S., & Mysliwiec, M. (2010 December). Kidney injury molecule-1 correlates with kidney function in renal allograft recipients. Transplantation Proceedings, 42(10), 3957–3959.

[22] Fontanilla, J., & Han, W. K. (March 2011). Kidney Injury Molecule-1 (KIM-1) as an early detection tool for acute kidney injury and other renal diseases. Expert Opinion on Medical Diagnostics, 5(2), 161–173.