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Original Article

Urinary expression of acute kidney injury biomarkers in patients after RIRS: it is a prospective, controlled study

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Abstract: Objective: To evaluated the damage effects of retrograde intra-renal surgery (RIRS) on kidney tissue by measuring kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosaminidase (NAG), liver-type fatty acid binding protein (LFABP) expression. Material and methods: We enrolled thirty consecutive patients (Group 1) who underwent RIRS that presented with renal calculi size < 2 cm. Forty-seven control patients (Group 2) with no signs or symptoms of urogenital disease were also enrolled for this study. Patients urine KIM-1, NGAL, NAG, and LFABP and creatinine levels were determined before the surgery, 2 hours after the surgery, and 24 hours after the surgery. Results: Demographic data were established and found to be similar between the two groups. Two hours after the surgery KIM-1/Cr and NGAL/Cr levels had increased significantly in urine compared to levels before the surgery (P:0.04, P:0.02 respectively) and decreased 24 hours after the surgery. The NAG/Cr and LFABP/Cr levels did not change significantly after the surgery. Conclusion: According to acute kidney injury (AKI) markers, this study is suggests that RIRS is a safe method , KIM-1/Cr and NGAL/Cr levels were increased first 2 hours but returned to initial levels within 24 hours after the surgery.

Keywords: Retrograde intra-renal surgery, acute kidney injury, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, N-acetyl-β-D-glucosaminidase

Introduction

Acute kidney injury (AKI) is typically diagnosed by measuring serum creatinine but, serum creatinine does not allow for early detection of AKI due to its inability to identify early kidney damage, need for alternative biochemical markers. Recent report has investigated multiple ways to determine AKI through the evaluation of various biomarkers of renal injury [1-3].

The current literature suggests that the most effective biomarkers of AKI are kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosaminidase (NAG) and liver-type fatty acid binding protein (LFABP), which are expressed after ischemic and nephrotoxic AKI [4]. KIM-1 is a type 1 transmembrane glycoprotein containing a novel 6-cysteine immunoglobulin-like domain plus a threonine/serine and proline-rich domain characteristic of mucin-like *O*-glycosylated proteins. Recent reports suggest that human and animal kidney proximal tubule epithelial cells (pTECs) expressed high levels of KIM-1 after ischemic or toxic injury [5, 6]. NAG is a lysosomal enzyme that it is abundantly expressed in pTECs. Urinary NAG enzyme leakage from the proximal tubular cells into the tubular lumen [7]. It was found that urinary NAG can be identified in patients with established AKI from a control group, including both normal individuals and patients with urinary tract infection [8]. NGAL is AKI biomarker which the endogenous and molecular role in AKI remains unclear, however; it is believed to play a role in iron transportation. The NGAL has been used to identify AKI after pediatric cardiac surgery and after cardiopulmonary bypass [9]. Liver fatty acid binding protein (LFABP) is a protein that is normally expressed in the proximal tubules of the kidney, which has been observed to be elevated in ani-

Table 1. Patient Demographics and Baseline Characteristics of Renal Stones

	Group 1 (n: 30)	Group 2 (n: 47)	*p value*
Age	39.8 ± 18.9	40.5 ± 21.4	0.07
Sex			
Male	15 (50%)	25 (53%)	0.12
Female	15 (50%)	22 (46%)	0.16
Stone localization			
Right	18 (60%)		
Left	12 (40%)		
Stone size (mm^2)	119.5 ± 71.6		
Mean HU (Hounsfield units)	957 ± 32.6		
Operation time (min)	56.4 ± 16.4		
Amount of irrigation (cc)	740.5 ± 157.8		
Laser time (min)	24.5 ± 18.2		
Grade of hydronephrosis	None 0		
	Mild 6 (20%)		
	Moderate 14 (46%)		
	Severe 10 (33%)		

mal models of AKI. In a recent prospective study of children undergoing cardiac surgery, urine LFABP increased at 4 hours post-bypass [10]. Various studies proposed that KIM-1, NGAL, NAG, LFABP are promising biomarkers for diagnosing AKI and evaluating clinical improvement about kidney [4].

The European Association of Urology (EAU) guidelines recommend the use of less invasive modalities such as extracorporeal shockwave lithotripsy (ESWL) or retrograde intra-renal surgery (RIRS), for stones less than 2 cm [11]. Major technological progress has been achieved for RIRS, which is mainly used for the renal collecting system on the upper ureter [12].

It was reported that ESWL may induce tissue damage by causing inflammation, bleeding and hemodynamic impairment. KIM-1 and NAG levels significantly increased after ESWL and returned to baseline within 2 weeks after ESWL [13]. Until now, we have not had enough information on the damage of the kidney as a result of RIRS. Also, there is no study about the use of the urinary markers KIM-1, NGAL, NAG, LFABP after RIRS [14, 15]. In this study, we aimed to evaluate the damage effects of RIRS on kidney tissue by measuring KIM-1, NGAL, NAG, and LFABP expression.

Patients and methods

A prospective controlled study was performed between March and September 2013. This study was approved by the Institutional Review Board of the Dicle University Medical Faculty. All patients treated with RIRS gave their informed consent before the procedure and informed consent was also obtained from all other subjects preceding their participation in the study. We enrolled thirty-four consecutive adult patients who underwent RIRS (Group 1) with renal calculi area less than 2 cm. Four patients were excluded from study because DJ stent was pleaced for residual stone. Subjects who had previous renal surgery, ESWL, a solitary kidney, congenital renal anomalies, comorbid disease as diabetes mellitus, hypertension or evidence of other urinary tract disorders such as pyelonephritis were excluded from the study.

Forty-seven control patients (Group 2) with no known urogenital disease and no known history of urologic pathology participated in this study. Volunteers taken blood sample for creatinine and provided one freshly voided urine sample when they were fasting.

Patients demographic data were collected as age, sex, operation time, amount of irrigation fluid, stone localization and stone size (**Table 1**). Pre-operative tests, such as blood routine tests, serum biochemistry, urinalysis, urine culture, ultrasonography, and plain X-ray were recorded. Computerized tomography (CT) was done routinely, in order to analyze the size and location of the stone as well as the anatomical structure. Pre-operative antibiotics were administered based on the results of the urine culture. RIRS was performed using a flexible ureterorenoscope (size 3.7 F with a 270° angle deflection) (Karl Storz, Endoscopes, Culver City, CA, USA). The stones were fragmented with a holmium (Ho: YAG) laser until they were deemed small enough to pass spontaneously.

Patient urine creatinine levels and AKI markers were measured on the pre-operative day, the

Table 2. Early renal injury markers in patients with urolithiasis who underwent retrograde intrarenal surgery (RIRS)

	Controls (n = 47)	Pre-op (n = 30)	Post-op 2 h (n = 30)	Post-op 24 h (n = 30)
Kim 1/Cr	1.86 ± 1.29	2.24 ± 1.14	5.16 ± 2.18	2.42 ± 1.60
NGAL/Cr	557 ± 316	575 ± 215	940 ± 171	608 ± 296
NAG/Cr	0.13 ± 0.04	0.11 ± 0.08	0.16 ± 0.13	0.13 ± 0.09
LFABP/Cr	0.43 ± 0.17	0.41 ± 0.39	0.38 ± 0.32	0.40 ± 0.28

Table 3. Differences (*p* values) in the early kidney injury markers to creatinine ratio between the first measurements and the controls

Compared modalities	Kim 1/Cr	NGAL/ Cr	LFABP/ Cr	NAG/ Cr
Pre-op/Controls	NS*	NS*	NS*	NS*
Pre-op/Post-op 2 h	0.04	0.02	NS*	NS*
Pre-op/Post-op 24 h	NS*	NS*	NS*	NS*

*NS: Not significant (P > 0.05).

post-operative second hour (2 h), and post-operative first day (24 h) using an auto analyzer Architect C 16000 (Abbott Laboratories, Abbott Park, IL, USA). Urine KIM-1, NGAL, NAG, and L-FABP levels were determined using a commercially available quantitative sandwich immunoassay technique (SunRed Biotechnology Company, Shanghai, China). All activity of the enzymes tested were given as a ratio of biomarker to urine creatinine values for each patient.

Statistical analysis

For statistical analyses SPSS (SPSS Inc, Ca, Ill, USA) software package program was used. Mean (± standard deviation), values of data were calculated. All the data were presented as mean ± SD. The distribution of the data was evaluated by the Kolmogorov-Smirnov test. The comparisons of the groups were performed using independent sample t-tests. The correlation between operation time, amount of irrigation, laser time and AKI markers was tested by Pearson's correlation test. A *p*-value less than 0.05 was accepted as the cut-off value for the level of statistical significance.

Results

Demographic data were comprised of patient group 1 [(n = 30), 15 were males and 15 were females)] and a control group 2 [(n = 47), 25 were males and 22 were females)]. The mean age of patients was 39.8 ± 18.9 and 40.5 ± 21.4 years in group 1 and group 2, respectively (P = 0.07). The mean stone size was 119.5 ± 71.6 mm^2 in group 1. Demographic data were found to be similar between the two groups. There was no residual stones (> 4 mm) in the kidney and all patients obstruction was resolved. The mean levels of KIM-1, NGAL, NAG, and LFABP is summarized in **Table 2** and the statistical comparison of the ratio to creatinine levels as same time is shown in **Table 3**. The mean levels of KIM 1/Cr did not differ in the study group of pre-operative patients when compared to those of control. KIM 1/Cr mean levels were presented for before the surgery, 2 h after the surgery, and 24 h after the surgery as 2.24 ± 1.14, 5.16 ± 2.18, 2.42 ± 1.60, respectively. Two hours after the surgery KIM-1/Cr level had increased significantly in urine (P:0.04) and returned the initial levels 24 hours after the surgery. The mean levels of NGAL/Cr were 575 ± 215, 940 ± 171, 608 ± 296 for the different operative time points. NGAL/Cr mean levels increased significantly 2 h after the surgery (P:0.02) and returned to initial levels of NGAL the 24 h after the surgery The mean levels of the NAG/Cr that pre-operative, post-operative 2 h, and post-operative 24 h were 0.11 ± 0.08, 0.16 ± 0.13, 0.13 ± 0.09, respectively. The NAG/Cr levels did not increase significantly after the operation. The mean levels of LFABP /Cr levels were 0.41 ± 0.39, 0.38 ± 0.32, 0.40 ± 0.28, respectively and also did not change significantly after the RIRS. The relationship between operation time, amount of irrigation, laser time and AKI markers 2 h after surgery listed in **Table 4**. Kim 1/Cr levels were significantly correlated with the amount of irrigation. NGAL/Cr levels were significantly correlated with operation time and amount of irrigation.

Discussion

RIRS has been used successfully to access and treat complex renal calculi smaller than 2 cm with high reported stone-free rates [12]. It has fewer overall complications compared to PCNL, with a high absence of renal injury. However, the small working channels of RIRS had limited the usefulness of effective instrumentation

Table 4. Correlation between operation time, amount of irrigation, grade of hydronephrosis and AKI markers level 2 h after RIRS

	Kim 1/Cr Post-op 24 h		NGAL/Cr Post op 24 h		LFABP/Cr Post op 24 h		NAG/Cr Post op 24 h	
	r	p	r	p	r	p	r	p
Operation time (min)	0.210	NS	*0.836*	*< 0.05*	0.220	NS	0.446	NS
Amount of irrgation (cc)	*0.857*	*< 0.05*	*0.910*	*< 0.01*	0.316	NS	0.412	NS
Laser time (min)	0.346	NS	0.642	NS	0.436	NS	0.528	NS

r: Pearson correlation coefficient. NS: Not significant (P > 0.05).

that allows concurrent stone fragmentation and removal [16]. RIRS for kidney stone treatment is known as a safe, minimally invasive method and does not directly damage the kidney because it is a retrograde process that is conducted using a flexible device that is able to move freely in the kidney. To what extent the kidney is affected during the process of renal parenchyma in studies based on subjective data is limited. The holmium laser that is used during the procedure is recognized as a mode urinary stones fragmentation [17]. Lasers produce a cavitation vapor bubble at the water-stone interface due to direct absorption of the laser energy in the water. The pulse duration also results in acoustic effect that aids stone fragmentation and destruction [18]. Creatinine has been used a long time for kidney damage, but in showing early response has been inadequate. We thought kidney injury molecules could help us in this regard. During the operation the increase in pressure of the kidney depends on irrigation or holmium laser energy can have a damaging effect on the kidney. Recently, several biomarkers have been explored for the early diagnosis of AKI, as KIM-1, NGAL, NAG and LFABP [19]. It has been reported that KIM-1 levels increase in multiple types of injury, including tubular, interstitial, and glomerular and were associated with the severity of kidney injury [20]. It was found a positive correlation between the degree of scarring and KIM-1 levels in patients diagnosed with vesicoureteral reflux [21]. In this study, it was observed significantly higher levels of the KIM-1/Cr 2 h after the surgery but 24 h after the surgery KIM-1/Cr levels returned to pre-operative levels. The results of the control group values and pre-operative values of patients with kidney stones were similar, not significantly different. It was found positive correlation between Kim/Cr and amount of irrigation 2 h after the surgery. NGAL protein is easily detected in the blood and urine soon after AKI in animal models [22]. NGAL has also been evaluated as a biomarker of AKI in kidney transplantation [23]. Several investigators have examined the role of NGAL as a predictive biomarker of nephrotoxicity following contrast administration, with promising results [24]. In this study, patients NGAL/Cr levels were increased significantly 2 h after the surgery and returned initial level 24 h after surgery. It was found positive correlation between NGAL/Cr with operation time and amount of irrigation. NAG is a lysosomal enzyme is located in proximal tubules. Therefore, increased activity of this enzyme in the urine may suggest injury to tubular cells and could serve as a specific urinary marker for tubular cell function [7]. Increased urinary NAG excretion has been reported in acute renal disease of varying etiology, namely induced by toxic agents, after cardiac surgery and renal transplantation [25, 26]. NAG/Cr levels did not change after the surgery. L-FABP is a protein expressed in the proximal tubule of the kidney. It was found positive correlation between NAG/Cr with operation time and amount of irrigation. In patients with septic shock and AKI, urinary L-FABP measured at admission was significantly higher in the control group than in the survivors [27]. In this study, L-FABP/Cr value has been found to be stable in urine for extended periods of time, and not changed urinary levels significantly. The Kim-1/Cr, NGAL/Cr values showed a significant increase 2 h after the surgery. This can be partially explained by the fact that excess pressure was generated in the kidney or holmium laser energy. In this study it was found positive correlation between NGAL/Cr, Kim-1/Cr with amount of irrigation. These potential biomarkers may be used as noninvasive diagnostic tests for the identification of kidney injuries. Additional studies are necessary before biomarkers may be used in routine clinical prac-

tice. Limitations in our study is that a critical range of these biomarkers are uncertain.

Conclusion

We aimed to determine the effect of RIRS on kidney damage and used potential AKI biomarkers levels as diagnostic indicators. In the future, we hope to use these biomarkers to compare the different stone surgery techniques and evaluate their role in kidney tissue damage after operation. Our data suggests that RIRS is a safe method, some AKI markers were increased first 2 hours but returned to initial levels after 24 hours.

Disclosure of conflict of interest

None.

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References

[1] Murray PT, Devarajan P, Levey AS, Eckardt KU, Bonventre JV, Lombardi R, Herget-Rosenthal S and Levin A. A framework and key research questions in AKI diagnosis and staging in different environments. Clin J Am Soc Nephrol 2008; 3: 864-868.

[2] Lameire N, Van Biesen W and Vanholder R. Acute renal failure. Lancet 2005; 365: 417-430.

[3] Bellomo R, Kellum JA and Ronco C. Defining acute renal failure: physiological principles. Intensive Care Med 2004; 30: 33-37.

[4] Devarajan P. Biomarkers for the early detection of acute kidney injury. Curr Opin Pediatr 2011; 23: 194-200.

[5] Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL and Sanicola M. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998; 273: 4135-4142.

[6] Vaidya VS, Ramirez V, Ichimura T, Bobadilla NA and Bonventre JV. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. Am J Physiol Renal Physiol 2006; 290: F517-529.

[7] Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV and Jaber BL. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007; 18: 904-912.

[8] Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P and Bonventre JV. Urinary biomarkers in the early diagnosis of acute kidney injury. Kidney Int 2008; 73: 863-869.

[9] Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J and Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005; 365: 1231-1238.

[10] Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P, Noiri E and Devarajan P. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 2008; 73: 465-472.

[11] Türk C, Knoll T, Petrik A, Sarica K, Seitz C, Straub M. Guidelines on urolithiasis: European Association of Urology 2010. Available from: http://www.uroweb.org/gls/pdf/Urolithiasis%202010.pdf%A0.

[12] Bryniarski P, Paradysz A, Zyczkowski M, Kupilas A, Nowakowski K and Bogacki R. A randomized controlled study to analyze the safety and efficacy of percutaneous nephrolithotripsy and retrograde intrarenal surgery in the management of renal stones more than 2 cm in diameter. J Endourol 2012; 26: 52-57.

[13] Evan AP, Willis LR, Lingeman JE and McAteer JA. Renal trauma and the risk of long-term complications in shock wave lithotripsy. Nephron 1998; 78: 1-8.

[14] Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol 2006; 17: 1503-1520.

[15] Nickolas TL, Barasch J and Devarajan P. Biomarkers in acute and chronic kidney disease. Curr Opin Nephrol Hypertens 2008; 17: 127-132.

[16] Marguet CG, Springhart WP, Tan YH, Patel A, Undre S, Albala DM and Preminger GM. Simultaneous combined use of flexible ureteroscopy and percutaneous nephrolithotomy to reduce the number of access tracts in the management of complex renal calculi. BJU Int 2005; 96: 1097-1100.

[17] Mulvaney WP and Beck CW. The laser beam in urology. J Urol 1968; 99: 112-115.

[18] Hale GM and Querry MR. Optical Constants of Water in the 200-nm to 200-microm Wavelength Region. Appl Opt 1973; 12: 555-563.

[19] Han WK and Bonventre JV. Biologic markers for the early detection of acute kidney injury. Curr Opin Crit Care 2004; 10: 476-482.

[20] Chiusolo A, Defazio R, Zanetti E, Mongillo M, Mori N, Cristofori P and Trevisan A. Kidney injury molecule-1 expression in rat proximal tubule after treatment with segment-specific nephrotoxicants: a tool for early screening of potential kidney toxicity. Toxicol Pathol 2010; 38: 338-345.
[21] Toker A, Ziypak T, Orsal E, Laloglu E, Bedir F and Aksoy Y. Is urinary kidney injury molecule-1 a noninvasive marker for renal scarring in children with vesicoureteral reflux? Urology 2013; 81: 168-172.
[22] Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, Schmidt-Ott KM, Chen X, Li JY, Weiss S, Mishra J, Cheema FH, Markowitz G, Suganami T, Sawai K, Mukoyama M, Kunis C, D'Agati V, Devarajan P and Barasch J. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 2005; 115: 610-621.
[23] Mishra J, Ma Q, Kelly C, Mitsnefes M, Mori K, Barasch J and Devarajan P. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol 2006; 21: 856-863.
[24] Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS and Dobrzycki S. Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. Am J Nephrol 2006; 26: 287-292.
[25] Bernard AM, Vyskocil AA, Mahieu P and Lauwerys RR. Assessment of urinary retinol-binding protein as an index of proximal tubular injury. Clin Chem 1987; 33: 775-779.
[26] Price RG. The role of NAG (N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992; 38 Suppl 1: S14-19.
[27] Doi K, Noiri E, Maeda-Mamiya R, Ishii T, Negishi K, Hamasaki Y, Fujita T, Yahagi N, Koide H, Sugaya T and Nakamura T. Urinary L-type fatty acid-binding protein as a new biomarker of sepsis complicated with acute kidney injury. Crit Care Med 2010; 38: 2037-2042.