The Effect of Magnetic Resonance Imaging and Computerized Tomography on Urinary Neutrophil Gelatinase Associated Lipocalin Levels

Manyetik Rezonans Görüntüleme ve Bilgisayarlı Tomografi Yöntemlerinin İdrarda Nötrofil Jelatinaz ile İlişkili Lipokalin Düzeylerine Etkisi

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ABSTRACT Objective: Urinary neutrophil gelatinase associated lipocalin (NGAL) is among novel markers assessed for early diagnosis of acute kidney injury. The effect of gadolinium-based contrast media on urinary NGAL concentrations has not been studied before. The aim of our study was to compare the effect of iodinated contrast agents and gadolinium-based contrast media on renal function and on urinary NGAL levels in patients with normal renal function. **Material and Methods:** The study group consisted of 40 patients. They were selected among patients who would undergo computerized tomography (CT) (n=20) or magnetic resonance imaging (MRI) (n = 20) scan. Patients with diabetes, malignancy or infection were excluded. Blood samples were drawn before, and 2 and 48 h after the procedure. Urine samples were collected before and 2 h after the procedure for NGAL measurements. Levels of urinary NGAL and blood creatinine before and after the procedure were compared. **Results:** Urinary NGAL levels did not significantly change 2 h after the procedure compared to baseline value at CT [29 (4.1 - 499) vs. 26.7 (1 - 113) ng/ml, p=0.100] and at MRI [16 (6.7 - 169) vs. 15.7 (3.5- 112) ng/ml, p=0.313]. Although creatinine levels at 48 h were higher compared to baseline levels, they did not increase up to 50% in the CT group. The serum creatinine did not change at any time point in the MRI group. **Conclusion:** Gadolinium or iodinated contrast agents did not significantly change urinary NGAL levels when used intravenously in patients with normal renal function.

Key Words: Acute kidney injury; tomography, X-ray computed; magnetic resonance imaging; LCN2 protein, human

ÖZET Amaç: İdrarda nötrofil jelatinaz ile ilişkili lipokalin (NJİL), akut böbrek hasarının erken tanısı için üzerinde çalışılan göstergelerden biridir. Gadolinum içeren kontrast maddelerin idrar NJİL düzeyleri üzerindeki etkisi daha önce değerlendirilmemiştir. Çalışmamızın amacı, böbrek fonksiyonları normal olan hastalarda iyotlu kontrast maddelerle, gadolinum içeren kontrast maddelerin böbrek fonksiyonları ve idrar nötrofil jelatinaz ile ilişkili lipokalin düzeyleri üzerindeki etkilerini karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışma grubuna 40 hasta alındı. Hastalar bilgisayarlı tomografi (n=20) ve magnetik rezonans görüntüleme (n=20) uygulanacak hastalar arasından seçildi. Malinite, diyabet veya enfeksiyonu olan hastalar çalışmaya alınmadı. Kan örnekleri işlemden önce ve işlemden 2 ve 48 saat sonra alındı. İdrar örnekleri ise işlemden önce ve 2 saat sonra toplandı. İşlemden önce ve sonra idrar NJİL ve kan kreatinin düzeyleri karşılaştırıldı. **Bulgular:** Gerek bilgisayarlı tomografide [sırasıyla 29 (4,1 - 499) ile 26,7 (1 - 113) ng/ml, p=0,100], gerekse manyetik rezonans görüntülemede [sırasıyla 16 (6,7 - 169) ile 15,7 (3,5- 112) ng/ml, p=0,313] idrar NJİL düzeyleri, işlemlerden 2 saat sonra, bazal değere göre herhangi bir değişiklik göstermedi. Bilgisayarlı tomografi grubunda kreatinin düzeyleri 48 saat sonunda bazal değerle kıyaslandığında daha yüksekti ama bu artış %50 oranında değildi. Manyetik rezonans görüntüleme grubunda kreatinin değerlerinde bir değişiklik olmadı. **Sonuç:** Böbrek fonksiyonları normal olan hastalarda intravenöz olarak kullanılan gadolinum veya iyotlu kontrast maddeler idrar NJİL düzeylerini anlamlı derecede değiştirmemektedir.

Anahtar Kelimeler: Akut böbrek hasarı; tomografi, X-ray bilgisayarlı; manyetik rezonans görüntüleme; LCN2 protein, insan

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Imaging studies using computerized tomography (CT) and magnetic resonance imaging (MRI) play a critical role in diagnosis, disease characterization and treatment planning. These procedures require an injection of iodinated or gadolinium based contrast media (GBCM). Although routinely used in clinical practice, iodinated contrast media and to a less extent GBCM possess side effects. Contrast-induced nephropathy is associated with iodinated contrast agents and nephrogenic systemic fibrosis with GBCM.[1] Gadolinium-based contrast agents, which were originally considered non-nephrotoxic, have been recommended to replace iodinated contrast media in patients at risk for acute renal failure. However, it has recently been reported to be associated with acute kidney injury.[2] On the other hand, nephrogenic systemic fibrosis is shown especially in patients undergoing dialysis or patients with glomerular filtration rate less than 30 ml/min.[3]

The incidence of acute kidney injury (AKI) is increasing to epidemic proportions. Development of AKI portends excessive morbidity and mortality. Delay in the diagnosis of AKI using conventional biomarkers like urine output and serum creatinine has been one of the important obstacles in implementing effective early interventions. Radiocontrast agents are among the causes of AKI and more patients than previously are undergoing contrast-based procedures. Although serum creatinine is used for the diagnosis of AKI, it is insensitive and is an unreliable biomarker during acute changes in kidney function. Serum creatinine concentrations can vary widely with age, gender, muscle mass, muscle metabolism, medication and hydration status. During acute changes in glomerular filtration, serum creatinine concentration does not accurately depict kidney function until steady state equilibrium has been reached. The serum creatinine concentration does not increase until about half of the kidney function is lost.[4]

Several biomarkers are being evaluated for early diagnosis of AKI and Neutrophil gelatinase associated lipocalin (NGAL) is one of the promising markers. It is a protein of lipoprotein family and is composed of 8 ß-strands. It is a polypeptide chain of 178 amino acids with a molecular mass of 25 kDa. Neutrophil gelatinase associated lipocalin is expressed by neutrophils and other epithelial cells including proximally convoluted tubule and gene expression is demonstrated in various human tissues like uterus, prostate, salivary gland, lung, trachea, stomach, colon and kidney.[5-7] It is a secreted tubular protein that enters both urine and serum rapidly after the onset of AKI. Several investigators have examined the role of NGAL as a predictive biomarker of nephrotoxicity following contrast administration.[8-10]

The effect of GBCM on urinary NGAL concentrations has not been studied before. On the other hand, previous studies included use of intraarterial iodinated contrast agents in patients undergoing angiography. Compared with iodinated contrast agents, GBCM are suggested to be non-nephrotoxic and are preferred in high-risk patients. However, this issue is still not clear. In our study, we evaluated the effect of iodinated contrast agents and GBCM on u-NGAL levels, one of the early AKI markers, in patients with normal renal function.

MATERIAL AND METHODS

PATIENTS

The study group consisted of 40 patients. They were selected among patients undergoing CT (n=20) or MRI (n = 20) scan. This study was approved by the Başkent University Institutional Review Board and was run in accordance with the Declaration of Helsinki. It was supported by the Başkent University Research Fund. All patients provided written informed consent. Gadolinium 0,2ml/kg (Gadoterat meglumine) and iodinated contrast agent 1,5 ml/kg (ioversol, which is a non-ionic low osmolality contrast agent) were used intravenously.

SPECIMEN CHARACTERISTICS

Ten milliliters of urine was collected from each patient at baseline and 2h after the procedure for urinary creatinine and NGAL measurements. The urine samples were centrifuged at 1000 g for 5 minutes and the supernatants were stored at –70°C.

ASSAY METHODS

Urinary NGAL level was determined by ARCHITECT *i*1000 analyzer (Abbott Laboratories, Abbott Park, IL, USA), which is a two-step (sandwich) assay using Chemiluminescent Microparticle Immunoassay technology. Serum and urine creatinine concentrations were measured by the Jaffé assay using Roche/Hitachi 912 systems (Roche Diagnostics, IN, USA). Complete blood count and serum lipid profile were also studied.

STUDY DESIGN

Patients with diabetes, malignancy or infection were excluded to eliminate confounding factors. All patients gave informed consent to participate in the study. Preventive fluid administration or N acetylcysteine use was avoided since patients with normal renal function were evaluated. Blood samples were drawn at baseline, and 2 and 48 h after the procedure for serum creatinine measurements. Kidney function was measured according to the Modification of Diet in Renal Disease formula. Contrast-induced nephropathy was defined as an at least 50% increase in creatinine levels from baseline.[11]

STATISTICAL ANALYSIS METHODS

Data were analyzed using SPSS software 9.05. For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro-Wilk tests and by histograms. Comparisons between groups were done with the Student's T test for normally distributed data and Mann-Whitney U test was used for data not normally distributed. Chi-square test was used to compare gender. Continuous variables were reported as mean ± standard deviation (SD) in parametric tests and median (interquartile) in nonparametric test. Wilcoxon signed rank test was used for analysis of the two quantitative data, u-NGAL levels before and after procedure. P values <0.05 was considered statistically significant.

TABLE 1: Clinical and laboratory characteristics of the patients.

	CT	MRI	p
N (F/M)	20 (8/12)	20 (9/11)	1.000
Age (years)	52.3±12.32	54.8±11.19	0.343
BMI (kg/m^2)	25.8±2.36	27.6±2.39	0.050
Creatinine (mg/dl)	0.81 (0.60-1.20)	0.80 (0.61-1.00)	0.378
MDRD GFR (ml/min)	89±16.04	93.35±15.88	0.448
Glucose (mg/dl)	98.55±6.64	97.05±8.55	0.569
Uric acid (mg/dl)	4.8±1.2	5.7±1.4	0.068
LDL cholesterol (mg/dl)	108.07±29.67	94.44±20.88	0.168
Triglyceride (mg/dl)	147.35±52.11	151.15±76.34	0.882
Hemoglobin (g/dl)	12.6±1.19	13.2±1.28	0.119
WBC (K/mm^3)	7.8±2.2	7.7±2.23	0.795

CT: Computerized tomography; BMI: Body mass index; GFR: Glomerular filtration rate; LDL: Low density lipoprotein; MDRD: Modification of diet in renal disease; MRI: Magnetic resonance imaging; WBC: White blood cell. Continuous variables reported as mean ± standard deviation or median (interquartile).

RESULTS

Clinical and biochemical characteristics were shown in Table 1. Overall 40 patients were included in the study etiher in the CT (n = 20, mean age 52.3 ± 12.32 years, 8 female) or MRI (n = 20, mean age 54.8 ± 11.19 years, 9 female) groups. All patients had normal renal function prior to contrast administration. No differences were noted between groups with respect to age and gender. Levels of serum creatinine and u-NGAL before and after contrast medium administration were shown in

TABLE 2: Values of serum creatinine, urinary NGAL before and after contrast medium administration.[a]

	CT (n = 20)			MRI (n = 20)		
	Pre	Post	p-value	Pre	Post	p-value
Creatinine 0 (mg/dl)	0.81 (0.60-1.20)			0.80 (0.61-1.00)		
Creatinine 2 (mg/dl)	0.81 (0.60-1.20)	0.89 (0.60-1.30)	0.546	0.80 (0.61-1.00)	0.81 (0.61-1.10)	0.247
Creatinine 48 (mg/dl)	0.81 (0.60-1.20)	0.93 (0.62-1.4)	0.017	0.80 (0.61-1.00)	0.81 (0.69- 1.00)	0.061
u-NGAL 0 (ng/ml)	29 (4.1-499)			16 (6.7-169)		
u-NAGL 2 (ng/ml)	29 (4.1-499)	26.7 (1-113)	0.100	16 (6.7-169)	15.7 (3.5- 112)	0.313

CT: Computerized tomography; MRI: Magnetic resonance imaging; u-NGAL: Urinary neutrophil gelatinase associated Lipocalin.

[a]Continuous variables were reported as median (interquartile).

Table 2. Creatinine levels at 48 h were higher compared to baseline levels in the CT group (0.81 (0.60 - 1.20) mg/dl vs. 0.93 (0.62 - 1.4) mg/dl, p= 0.017). However, contrast-induced nephropathy, defined as at least 50% increase in serum creatinine from baseline, developed in one patient in the CT group. The serum creatinine did not significantly change at any time point in the remaining patients. Urinary NGAL levels did not significantly change 2 h after the procedure compared to baseline value for CT [29 (4.1 - 499) vs. 26.7 (1 - 113) ng/ml, p= 0.100] and MRI [16 (6.7 - 169) vs. 15.7 (3.5 - 112) ng/ml, p= 0.313]. The ratios of u-NGAL/urinary creatinine were not different before and 2 h after.

DISCUSSION

This is the first study describing the u-NGAL variability at baseline and after CT or MRI procedures in a cohort of outpatient adults with normal renal function. We did not find a significant change in the levels of u-NGAL 2 h after the procedure compared to baseline.

Radiocontrast agents are considered among the causes of AKI. Acute deterioration in renal function caused by radiographic contrast agents is generally mild and transient but the risk is proportionally increased in patients with preexisting renal failure, long standing diabetes, heart failure, hypotension, and low hematocrit levels.[12] Recognition of renal dysfunction at an early stage allows the implication of preventive measures. Neutrophil gelatinase associated lipocalin is a promising marker for early detection of AKI. Because of its small molecular size (25 kDa) and resistance to degradation, NGAL is excreted and is detected in the urine. Urinary and serum NGAL levels were suggested as an early predictive marker of AKI in patients undergoing coronary angiography and following surgical procedures (cardiopulmonary bypass, kidney transplantation).[8-10,13-16] In those studies urine and serum levels of NGAL were reported to be higher after the procedures compared to baseline measurements. In the study of Hirsch *et al.*, significant elevation of NGAL concentrations in urine were noted within 2 h after contrast administration in the contrast-induced nephropathy group.[8] There was a small decrease in the non-contrast induced nephropathy group. That study included children with congenital heart disease undergoing angiography with contrast administration. Malyszko *et al.* showed similar results in adult patients with or without diabetes undergoing cardiac catheterization. Neutrophil gelatinase associated lipocalin was significantly elevated in urine after 4, 8 and 24 h, and in serum after 2, 4 and 8 h.[17] Bachorzewska-Gajewska *et al.* evaluated urinary and serum NGAL levels at baseline and after 2, 4, 12, 24, and 48 hours following percutaneous coronary intervention in patients with normal serum creatinine. They found a significant rise in serum NGAL levels after 2 and 4 h, and in urinary NGAL levels after 4 h, but serum creatinine did not change significantly during their study period.[10] Wagener *et al.* evaluated urinary NGL levels in patients with cardiac surgery[13] and showed increased levels of urinary NAGL immediately after the termination of cardiopulmonary bypass in patients with and without AKI. However, within an hour after surgery, while urinary NGAL concentration decreased in non-AKI patients, it continued to increase in patients who developed AKI. There was a decrease in urinary NGAL levels in our study also, but the difference was not statistically significant. Studies reporting increased levels of urinary or serum NGAL levels generally included patients who had undergone cardiac angiography. Those patients may experience procedural complications that can affect renal perfusion, such as fluid restriction, arrhythmias, myocardial infarction, hypotension and hemorrhage. Such complications do not occur to the same degree after intravenous contrast injections.[18] This may be attributed to factors associated with cardiac angiography but not with peripheral aministration of contrast agents. Preda *et al.* studied the effect of an iso-osmolar contrast agent on renal function in patients with monoclonal gammopathies and in patients with oncological disease[19] and found no statistically significant difference in serum creatinine, creatinine clearance and urinary NGAL levels 2 h after the procedure compared to baseline. On the other hand, a review showed the paucity of controlled

clinical studies that demonstrate renal damage caused by intravenous contrast agents. Because, most of the studies did not compare the incidence of post-contrast renal dysfunction with a matched controlled group of patients who did not receive contrast agents. [20] The absence of control groups and the extrapolation of data obtained from cardiac angiography to patients receiving intravenous contrast material may have led to an overestimation of the risk of contrast nephropathy.

Small sample size, evaluation of only two levels of urinary NGAL and lack of comparison o urinary NGAl levels with serum NGAL levels were the major limitations of our study. Previous studies reported an increase after contrast administration and that started at 2 or 4 hours and NGAL levels continued to increase subsequently. Thus, we chose to measure the levels at 2 h. It is difficult to draw blood frequently in outpatients. To evaluate the net effect of contrast agents, we recruited patients with no high risk and with normal serum creatinine levels. The results could be different in patients with diabetes, chronic kidney disease or other high-risk groups.

In conclusion, intravenous contrast agents do not have any significant effect on renal function in contrast to intra-arterial agents in patients with normal serum creatinine.

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