ORIGINAL ARTICLE

The role of Neutrophil Gelatinase-Associated Lipocalin in identifying contrast induced nephropathy development in the emergency department

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Abstract

Objective: To evaluate the diagnostic significance of neutrophil gelatinase-associated lipocalin in detecting the development of contrast-induced nephropathy in patients undergoing contrast imaging in an emergency department setting.

Methods: The case-control study was conducted at the emergency department of Uludag University, Turkey, between January 1 and July 1, 2012, and comprised patients who underwent a diagnostic thoracic or abdominal Computed Tomography examination with contrast agent. At 2 hours and 72 hours after the scan, control urea, creatinine, and neutrophil gelatinase-associated lipocalin values were recorded. Plasma lipocalin measurement was performed using fluorescence-detected immunoassay method. An increase in serum creatinine of more than 0.5 mg/dl or 25% elevation from the basal level was considered to be a marker for the occurrence of contrast-induced nephropathy. SPSS 13 was used for statistical analysis.

Results: Of the 80 subjects in the study, 60(75%) were cases and 20(25%) were controls. Contrast-induced nephropathy did not develop in any of the patients, and, accordingly, no significant increase of plasma urea, creatinine, or neutrophil gelatinase-associated lipocalin levels was observed. A significant positive relationship was found between urea and creatinine levels at 2 hours ($p<0.009$) and at 72 hours ($p<0.001$).

Conclusions: Diagnostic contrast computed tomography examination in patients with normal renal function did not lead to Contrast-induced nephropathy or increased neutrophil gelatinase-associated lipocalin levels, an accepted early indicator of kidney injury.

Keywords: Contrast induced nephropathy, Emergency department, NGAL. (JPMA 64: 1109; 2014)

Introduction

Contrast-induced nephropathy (CIN) is currently a major complication because of increasing usage of iodinated contrast media in procedures. CIN is the third most common cause of renal failure after impaired renal perfusion and the use of nephrotoxic medications.[1,2] In other studies, CIN incidence varied from 0% to 23% due to the differences in identification of kidney injury and comorbid conditions in patient populations.[3-5]

CIN develops after intravascular contrast medium administration and is diagnosed after ruling out all other potential causes of kidney injury. The most common significant indications of CIN are a rise in serum creatinine of 0.5mg/dl or a 25% increase from the baseline value, as assessed at 48-72h after the procedure.[6,7] Important risk factors for CIN include pre-existing renal insufficiency, diabetes mellitus, older age, volume of contrast medium administered, advanced heart failure, haemodynamic instability, dehydration, and concomitant use of nephrotoxic medications.[5,8-10] Incidence of CIN among individuals without risk factors is reported to be 3-5%, whereas CIN incidence rises to 100% in those who have four risk factors.[5]

Contrast media are grouped according to their osmolality or iconicity.[4,11,12] Toxic effects of contrast media are thought to depend primarily on the contrast medium's osmolality. Ionic contrast media have high osmolality (>1500 mOsm/kg H2O). On the other hand, non-ionic agents are not water soluble. Theoretically, the best imaging is achieved using non-ionic contrast agents, which have large amounts of iodine atoms and insoluble particles; these types of contrast agents have fewer nephrotoxic effects than ionic agents.[4,11] Furthermore, various studies have demonstrated that non-ionic contrast media with low osmolality lead to CIN.[4,11,13]

Two important mechanisms are considered to be responsible for the development of CIN. The first mechanism is a direct toxic effect of the contrast media, resulting in mitochondrial dysfunction in renal tubular cells, generation of reactive oxygen species (ROS), and apoptosis.[7,13] The second mechanism is the creation of an imbalance between tissue oxygen delivery and oxygen use, which occurs due to a reduction of blood flow in the

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medulla caused by contrast medium. Therefore, active sodium transport in the final portion of the proximal tubulus and in the thick ascending limb of the loop of Henle is affected.[14,15]

The diagnosis of CIN is restricted due to the lack of a specific and sensitive biomarker of kidney injury. Currently, markers such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1, and interleukin 18 are used to determine kidney injury.[16]

NGAL is a polypeptide chain of 178 amino acids that has a small size of 25-kDa and covalently bonds to neutrophil gelatinase. Normally, NGAL is present at low basal levels in various human cell types, tissues and organs, such as kidney, liver, stomach and colon.[17] NGAL has bacteriostatic, antioxidant and growth factor effects, as it affects cell proliferation, apoptosis and differentiation. Injured epithelial cells release NGAL, with levels increasing between 2 and 12 hours. Microanalysis results from animal experiments have indicated that NGAL is the earliest excreted protein after ischaemic or toxic injury to the kidney. NGAL protein can be easily distinguished using blood and urine samples in the case of acute kidney injury.[16,18] For this reason, NGAL can be employed as an early, sensitive and reliable biomarker in detecting acute kidney injury.[19]

The aim of the current study was to evaluate the diagnostic significance of NGAL in detecting the development of CIN in patients undergoing contrast imaging in an emergency department (ED).

Patients and Methods

After obtaining approval of the institutional ethics committee, the case-control study was conducted at Uludag University, Turkey, from January 1 to July 1, 2012, and comprised patients who underwent a diagnostic thoracic or abdominal computed tomography (CT) scan with contrast agent. Normal urea-creatinine level at admission was taken as the basis of inclusion in the study. As a control group, NGAL levels of 20 healthy volunteers were evaluated. The sample size achieved 88.538% power to detect a difference of -30.0 between the null hypothesis mean of 95.0 and the alternative hypothesis mean of 125.0 with a known standard deviation of 60.0 and with a significance level (alpha) of 0,05000. Exclusion criteria comprised age less than 18, diabetes mellitus, compensated renal failure, glomerular disease, renal transplantation, history of trauma, and history of contrast imaging within the preceding three days. During thoracic and abdominal CT scans with contrast agent, intravenous iopromide (Ultravist) 370/150 ml (ionic) contrast was administered. Two hours after the contrast CT scan, control urea-creatinine and NGAL levels were recorded. The patients who underwent diagnostic imaging and were discharged were asked to come back 72 hours later when urea-creatinine and NGAL levels were again measured. Blood samples from hospitalised patients were taken in their respective clinics 72 hours later. Plasma NGAL measurements were performed using a Triage® NGAL Test Device (Biosite Inc., San Diego, CA, USA) via a fluorescence-detected immunoassay method. This test uses a fluorescence immunoassay to measure NGAL levels in anti-coagulated blood or plasma samples. It also utilises micro-particles and anti-NGAL coated with monoclonal antibodies. The measurable NGAL range is 60-1300ng/ml. The reference interval upper limit is 149ng/ml with a 90% confidence interval (CI) (100-194ng/ml). Measurements above this level were accepted as significant for CIN development. An increase in serum creatinine of more than 0.5mg/dl or 25% following contrast administration was considered to be a marker for the occurrence of CIN. As a control group, NGAL levels of 20 healthy volunteers were evaluated.

Statistical analysis of the data was carried out using SPSS 13. Mann-Whitney U and Friedman tests were used to determine whether variables were significantly different from each other, and Spearman analysis was employed to determine whether there was a relationship between the variables.

Results

Of the 60 patients in the study, 28(46.6%) were female and 32(53.4%) were male. Overall, 21(35%) were between the ages of 18 and 50 years, and 39(65%) were over 50.

Table-1: Basal urea and creatinine levels in (mg/dl) at 2 hours and 72 hours.

	Urea				Creatinine			
	Basal	2-hours	72-hours	p-value	Basal	2-hours	72-hours	p-value
Median	35	34	33		0.8	0.8	0.7	
Minimum	14	11	13	0.388	0.1	0.1	0.5	0.794
Maximum	85	85	77		1.3	1.3	2.1	

*Comparisons were performed with Friedman test.

Table-2: Comparison of 2-hour and 72-hour urea, creatinine, and NGAL levels.

		2-hour urea	2-hour creatinine	2-hour NGAL	72-hour urea	72-hour creatinine	72-hour NGAL
2-hour urea	correlation coefficient	----	0.333** p=0.009	p=0.661			
2-hour creatinine	correlation coefficient	- 0.333 p=0.009	----	p=0.767			
2-hour NGAL	correlation coefficient	p=0.661	p=0.767	----			
72-hour urea	correlation coefficient				----	0.477*** p<0.001	p=0.067
72-hour creatinine	correlation coefficient				0.477*** p<0.001	----	p=0.427
72-hour NGAL	correlation coefficient				p=0.067	p=0.427	----

**Correlation coefficient: 0.333, p=0.009, ***Correlation coefficient: 0.477, p<0.001.
NGAL: Neutrophil gelatinase-associated lipocalin.

Table-3: Comparison of NGAL levels of control and patient groups.

	Patient	Control	p-value*
N	60	20	0.272
Median	70.50	78.65	
Minimum	60	60	
Maximum	764	135	

*Comparisions were performed with Mann-Whitney U test.
**2-hour of NGAL levels of patients group were considered.
NGAL: Neutrophil gelatinase-associated lipocalin.

Among the controls, there were 11(55%) females and 9(45%) males, and 14(70%) were between the ages of 18 and 50 years, while 6(30%) were over 50.

Of the patients, 41 (68%) underwent contrast thorax CT, while 19(32%) underwent contrast abdominal CT. None of the patients developed CIN.

There was no significant difference between basal urea levels and the 2- and 72-hour levels (p<0.388). Similarly, no difference was found between the 2- and 72-hour urea levels (Table-1). There was also not a significant difference between basal creatinine levels and the 2- and 72-hour levels, and there was not a difference between the 2- and 72-hour creatinine levels (p<0.794).

However, there was a significant positive relationship (p<0.009) between urea and creatinine levels at 2 hours, as well as between urea and creatinine levels at 72 hours (p<0.001) (Table-2).

There was not a significant association between NGAL levels and creatinine levels at 2 hours. Similarly, there was not a significant association between NGAL levels and urea or creatinine levels at 72 hours. No significant association was found between NGAL levels measured 2 hours after the application of contrast medium and urea or creatinine levels measured at 72 hours. Similarly, there was not a significant association between NGAL levels of the patients at 2 hours and their NGAL levels at 72 hours. No significant association was observed when comparing the 2- and 72-hour NGAL levels of the study group and those of the control group (Table-3).

Discussion

NGAL has emerged as a promising and commonly used biomarker for the early diagnosis and prediction of kidney injuries.[19] In recent years, it has also been employed in various disease groups, such as chronic obstructive pulmonary disease (COPD), heart failure, abdominal aortic aneurysm (AAA) etc.[20-22] It is now widely recognised that NGAL, whether measured in urine or plasma, is a valuable indicator and the earliest known responding marker of acute kidney injury.[2,19] In possible cases of renal function disorder due to iatrogenic causes, the function of NGAL becomes more of an issue.[16,19] The incidence of iatrogenic causes of kidney disorders has been shown to increase with frequent use of radio-diagnostic procedures, including contrast media.

Extensive cohort research performed on adults has demonstrated that CIN is the third most frequent cause of acute kidney injury acquired in the hospital and is responsible for 11-13% of all incidences. Approximately one-half of these incidences involved cardiac catheterisation and angiography, and approximately one-third involved CT examination.[1]

Several studies have researched angiography and the nephrogenic effects of contrast medium.[23,24] The objective of our prospective pilot study was to evaluate whether contrast medium imaging methods conducted in the ED for diagnostic purposes lead to CIN. Contrast CT is a frequently applied diagnostic method used in the ED to rule out life-threatening diseases. CT is used particularly for the distinctive fatal diagnoses associated with chest and abdominal pain. CIN occurs generally within 2-5 days following the administration of contrast medium. This period is risky for the patient, and progression of kidney function disorder may be prevented by close follow-up of the patient, including

avoiding nephrotoxic agents and ensuring optimal liquid intake.

NGAL is excreted from injured kidney epithelial cells, and NGAL levels increase within 2-12 hours following injury. Micro-analytical measurements in animal experiments have shown that NGAL was the earliest excreted protein following ischaemic or toxic injuries in the kidney. NGAL protein can be easily analysed in blood and urine samples in cases of acute kidney injury.[25]

In a study that addressed early detection of the development of CIN in patients with coronary angiography, it was observed that serum and urine NGAL levels rise 2-4 hours after the administration of contrast medium.[23,24] However, CIN did not develop in any of the patients with high NGAL levels. The authors stated that this result was not surprising, as NGAL levels increased with respect to the basal values, and therefore, the increase in NGAL levels in this study did not result in the development of CIN. In another study performed to investigate the early detection of CIN development following coronary angiography, it was observed that CIN developed in 13 out of 150 patients, and the increase in NGAL levels in those patients 24 hours after contrast medium administration was more significant than the increase in creatinine levels.[2]

In our study, urea and creatinine levels were measured 2 and 72 hours after the administration of contrast medium. While measurement at 2 hours may be considered too early, the purpose of this time point in the study was to identify any risk for CIN development in the patient so that necessary precautions could be taken, as NGAL levels begin to increase after 2 hours[2,19,23,24] in an acute kidney injury. This potentially early indication of CIN was desirable because some of the patients who had a contrast CT scan in the ED were discharged. In our study, 14 (23%) patients were discharged from the ED. Therefore, if the NGAL level measured after 2 hours was high, follow-up could be provided, and the necessary precautions and recommendations (sufficient hydration, protective treatments, avoidance of nephrotoxic agents, etc.) could be implemented. Diagnosis of contrast nephropathy requires increased levels of both NGAL and creatinine after 72 hours. In our study, CIN did not develop in any of the patients, and no significant increase was observed in the urea and creatinine levels of the patients. On the other hand, a significant positive correlation was observed between urea levels and creatinine levels at 2 hours ($p<0.009$), as well as between urea levels and creatinine levels at 72 hours ($p<0.001$). This result is not surprising because a homogenous patient population was used in the study. The only etiological factor for acute kidney injury in our patients was the administration of contrast medium. All patients had normal kidney functions. These patients did not have comorbid variables, such as diabetes mellitus, high blood pressure or nephrotoxic intake. All patients received the same amount of the same contrast medium, thus avoiding variability regarding the medium.

Changes in NGAL concentrations occurring after contrast medium administration were evaluated, and these changes were compared with those for serum urea and creatinine levels. This analysis allowed for the development of a gold standard for CIN identification. None of our patients developed CIN, and accordingly, a significant increase in NGAL levels was not detected.

In terms of limitations, it was a single-centre study with a small study population. Similar studies are needed on larger patient populations with comorbidities, especially diabetes mellitus.

Conclusion

Diagnostic contrast CT examination in patients with normal renal function did not lead to CIN development and did not cause an increase in NGAL levels, which is accepted as an early indicator of kidney injury.

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