Murat Muratoglu¹ MD
Cemil Kavalci¹, MD, Assoc. Prof.
Elif Kilicli¹, MD; Meliha Findik¹, MD
Afşin Emre Kayipmaz¹, MD
Polat Durukan², MD, Prof.

¹Başkent University Faculty of Medicine,
Department of Emergency Medicine, Ankara,
Turkey

²Erciyes University Faculty of Medicine,
Department of Emergency Medicine, Kayseri,
Turkey

Serum Neutrophil Gelatinase-Associated Lipocalin Levels In Early Detection Of Contrast-Induced Nephropathy

Abstract

Purpose: The purpose of this study was to investigate the role of serum neutrophil gelatinase-associated lipocalin (NGAL) levels in the early detection of contrast-induced nephropathy (CIN).

Methods: This prospective study enrolled 74 patients undergoing abdominal tomography with contrast (1 November 2014 - 28 February 2015). Demographic properties (age and sex), symptoms and CT examination results were analysed. Sodium, potassium, urea, creatinine and NGAL levels were measured at 0th, 6th, and 72nd hours. P value < 0.05 was considered statistically significant.

Results: CIN developed in 16.2% of the study patients. The mean age was significantly higher in the patients who developed CIN ($p < 0.05$). No significant correlation existed between the occurrence of CIN and patient gender ($p > 0.05$). Urea levels did not differ significantly between the groups at 0th and 6th hours ($p > 0.05$) but was significantly higher in the patients with CIN at 72nd hour ($p < 0.05$). Urea levels did not change significantly over time in the entire group ($p > 0.05$). Creatinine level was not significantly different between the groups ($p > 0.05$) but increased significantly over time ($p > 0.05$). There were no significant differences between the groups with respect to NGAL levels at 0th and 72nd hours ($p > 0.05$) whereas the group with CIN had a significantly higher NGAL level at 6th hour ($p < 0.05$). A NGAL level of 668 mg/dL at 6th hour had a sensitivity of 100%, specificity of 95%, positive predictive value of 80% and negative predictive value of 100% for the detection of CIN.

Conclusion: NGAL may be a useful marker for the early detection of CIN.

Manuscript submitted February 10th, 2016

Manuscript accepted April 27th, 2016

Clin Invest Med 2016; 38 (3): E88-E94.

Correspondence to:

Dr. Cemil Kavalci

Başkent University Faculty of Medicine, Department of Emergency Medicine,
Bahçelievler/Ankara/Turkey

E-mail: cemkavalci@yahoo.com

With technological advances, imaging modalities have gained an important place in the diagnostic protocols of certain disorders. Contrast agents, which increase the efficacy of imaging modalities, are currently used in imaging procedures of nearly 60 million cases a year worldwide [1]; unfortunately, their use has resulted in some allergic, cardiac and renal side effects [2]. Contrast-induced nephropathy (CIN) ranks third among in-hospital nephropathy cases [3].

Contrast-induced nephropathy is defined as a 0.5-1.0 mg/dL or 25-50% increase in creatinine level 48-72 hours after contrast administration [4]. Despite the recommendation to administer intravenous fluids, *N*-acetyl cysteine, ascorbic acid, and some vasodilatory agents, no method exists to reliably prevent CIN [5-7], although some research has been done with the aim of detecting acute renal failure (ARF) and CIN in a timely manner [8-10].

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein that is released in large amounts in ischemic renal injury in animal models [11]. Studies have demonstrated that NGAL is a marker having a high sensitivity and specificity for the prediction of acute renal injury [12,13]. Lee *et al.* demonstrated in an animal model that urinary NGAL level increased significantly earlier than serum creatinine level in the event of acute renal injury [8]. Padhy *et al.* reported that NGAL peaked at 4th hour and returned to normal by 48th hour in CIN after coronary angiography [14]. In a recent paper, Philipps *et al.* demonstrated that NGAL was a good marker of drug-induced acute renal injury [15].

Our goal in this study was to determine if serum NGAL levels might be helpful in early recognition of CIN.

Materials and Methods

Study design

This prospective, observational, cohort study was conducted at Başkent University Ankara Hospital, Department of Emergency Medicine between 1 November 2014 and 28 February 2015. A total of 74 consecutive patients were enrolled. The study was designed to determine the role of serum NGAL level in the early detection of contrast-induced nephropathy.

Patient selection

The inclusion criteria were as follows: an indication for CT examination with contrast; 18 years of age or older; no history of any renal disorder, DM, or the use of a nephrotoxic medication; no contrast allergy; and, informed consent for the participation in the study.

The exclusion criteria were as follows: younger than 18 years of age; a history of any renal disorder, DM, or the use of a nephrotoxic medication; a contrast allergy; and, refusing to provide consent for the participation in the study.

Study procedures

Demographic properties (age and sex), presenting complaint(s), urea, creatinine and NGAL levels and CT results were analysed. All patients received the prophylaxis (500 mL isotonic crystalloids like 0.9% saline) for prevention of contrast-induced acute kidney injury. All patients were administered Ioversol (Optiray) (equivalent of 359 mg/mL of iodine) intravenously at a dose of 1-1.5 mL/kg.

Measurements

Each patient gave a blood sample that is put into a biochemistry tube without added anticoagulant at 0th hour before contrast exposure (Sample 1) and at 6th (Sample 2) and 72nd hours (Sample 3) after contrast agent exposure. These blood samples were used to analyse serum urea nitrogen (BUN) and creatinine levels. The blood samples taken for the determination of NGAL levels were categorized into 0th, 6th, and 72nd hour groups after centrifugation. The samples were stored at or below -20°C until analysis. Serum NGAL levels were measured with the ELISA method.

Statistical analysis

Study data were analysed using SPSS Windows 18 software package. The descriptive statistics included mean±standard deviation, median, interquartile range (IQR) and frequency. Data distribution was analysed with the Kolmogorov Smirnov test. The study subjects were grouped into two as "CIN developers" (Group 1) and "CIN non-developers" (Group 2). Parametric variables were analysed with the Student's T test; non-parametric variables with the Mann Whitney-U test and the Kruskal-Wallis test, the qualitative variables with Chi-Square test and the temporal changes of the measurements with the Friedman Test. A Receiver Operating Characteristic (ROC) analysis was done to determine the sensitivity and specificity of NGAL for the detection of CIN. The study data were assessed in a confidence interval of 95%. A p level of <0.05 was considered statistically significant. The power of the study was calculated 0.80.

Ethical considerations

This study was approved by the Başkent University ethics committee and conducted at Başkent University Ankara Hospital, Department of Emergency Medicine in compliance with the Helsinki Declaration. A written informed consent was obtained either from the patients or from their relatives

Results

Patients

The mean age of the study population was 43.1 ± 15.1 years. Fifty-two (70.3%) patients were female and 22 (29.7%) were male. Seventeen (22.9%) patients were 60 years or older.

The most common admission symptoms were nausea (64.9%) and abdominal pain (66.2%). The distribution of the admission symptoms was presented on Table 1. The CT examination revealed a pathology in 36 (48.6%) patients. The most commonly observed pathology was acute appendicitis (36.1%). The distribution of the pathologies detected by abdominal CT was summarized on Table 2.

Twelve (16.2%) patients developed CIN. The patients who developed CIN had a mean age of 57.8 ± 12.4 years, while those who did not develop CIN had a mean age of 40.2 ± 14.0 years ($p < 0.05$) (Table 3). Ten (83.3%) of the patients with CIN were female and two (16.7%) were male. In the group without CIN, 42 (67.7%) patients were female and 20 (32.3%) were male. No significant correlation was found between the occurrence of CIN and patient gender ($p > 0.05$) (Table 3).

Biochemical measurements

Serum BUN, creatinine and NGAL levels of the two study groups are summarized in Table 3. While there was no significant differences between the groups in terms of serum urea level measured at 0th and 6th hours ($p > 0.05$), 72nd hour serum urea levels were significantly higher in the CIN group

($p < 0.05$) (Table 4). The same also applied for serum creatinine levels in that 0th and 6th hour creatinine levels were not different between the groups although 72nd hour serum creatinine levels differed significantly between the groups ($p < 0.05$) (Table 3). While there were no significant differences between the 0th and 12th hour serum NGAL levels of the two groups, the two groups had significantly different 6th hour serum creatinine levels ($p < 0.05$) (Table 3).

Table 4 summarizes the temporal changes in serum BUN, creatinine and NGAL levels in patients who developed CIN. The temporal changes of serum BUN, creatinine and NGAL levels were statistically significant ($p < 0.05$). Serum BUN and creatinine levels rose at the 72nd hour; NGAL level increased at the 6th hour and decreased at the 72nd hour.

ROC analysis was performed to determine the sensitivity and specificity of NGAL for the prediction of CIN development. A NGAL level of 668 mg/dL had a sensitivity of 100%, a specificity of 95%, a positive predictive value (PPV) of 80%, and a negative predictive value (NPV) of 100% (AUC= 0.98, CI= 0.91 – 0.99, Youden index J=0.95) (Figure 1).

Discussion

We demonstrated that NGAL level increased at the 6th hour and decreased at the 72nd hour. These data suggest that serum NGAL level increased in the acute stage of renal injury.

A major finding of our study is an early increase in NGAL level, compared with BUN and creatinine levels, in patients with CIN. While BUN and creatinine peaked at the 72nd hour, NGAL did so at the 6th hour. Thus, NGAL offers promise for the early diagnosis of CIN in the emergency department. Filiopoulos *et al.* reported that NGAL level at the 6th hour had a sensitivity and specificity of 100% [16]. Plasma

TABLE 1. Distribution of patients by admission symptoms

Admission symptom	Number	%
Abdominal pain	49	66.2
Nausea	48	64.9
Chest pain	23	31.1
Dyspnea	22	29.7
Vomiting	18	24.3
Back pain	17	23.0

TABLE 2. Distribution of the pathologies detected by CT

CT Result	Number	%
Acute Appendicitis	13	36.1
Ovarian Cyst Rupture	6	16.6
Acute Pneumonia	6	16.6
Pericardial Effusion	6	16.6
Pulmonary Thromboembolism	3	8.3
Mesenteric ischemia	1	2.7
Aortic dissection	1	2.7
Total	36	100

TABLE 3. Characteristics of patients according to groups

Variable		Group 1	Group 2	P value
Age (years) Mean $\pm$ SD		57.8 $\pm$ 12.4	40.2 $\pm$ 14.0	<0.001
Gender (number)	M/F	2/10	20/42	0.279*
BUN(mg/dl) Median(IQR)	0 th hour	13.5 (8)	12 (4)	0.164*
	6 th hour	15 (10)	11.5 (5)	0.135*
	72 nd hour	31 (18)	10 (5)	<0.001*
Creatinine (mg/dl) Median(IQR)	0 th hour	0.73 (0.17)	0.72 (0.13)	0.461*
	6 th hour	0.79 (0.13)	0.72 (0.11)	0.313*
	72 nd hour	1.36 (0.65)	0.75 (0.15)	<0.001*
NGAL(mg/dl) Median(IQR)	0 th hour	243.5 (556.8)	194.5 (295.8)	0.085*
	6 th hour	1050.5 (426.8)	233 (259.3)	<0.001*
	72 nd hour	307 (189.3)	196 (216)	0.69*

TABLE 4. Temporal change of the biomarkers in patients with CIN

	0 th Median (IQR)	6 th Median (IQR)	72 nd Median (IQR)	P
BUN	13.5 (8)	15 (10)	31 (18)	0.005**
Creatinine	0.73 (0.17)	0.79 (0.13)	1.36 (0.65)	0.000**
NGAL	243.5 (556.8)	1050.5 (426.8)	307 (189.3)	0.005**

*One-Way Anova Test, ** Friedman Test, IQR=Interquartile range

NGAL has been reported to be a highly specific and objective marker for the diagnosis of ARF [17-19]. In the current study, NGAL had an AUC level of 0.98, a sensitivity of 100% and a specificity of 95%. Since the determined AUC, sensitivity and specificity levels were quite high, NGAL may prove useful in the detection of CIN.

It has been shown previously that NGAL can be used for the diagnosis of acute renal injury [8,20-24]. Haase *et al.* showed that NGAL, measured 6 hours after patient admission, can be used as a marker to predict ARF [25]. Kavalci *et al.* demonstrated that NGAL level may be a predictive parameter for urgent haemodialysis [26]. Ozkan *et al.* stressed that serum NGAL levels may be used for both the detection of renal injury and the differentiation between acute and chronic renal injury [27]. Padhy *et al.* reported that NGAL peaked at 4th hour and returned to normal by 48th hour in CIN after coronary angiography [14]. Laçin *et al.* reported that NGAL level elevated at 24th hour and tended to decrease by two days

[9]. Filiopoulos *et al.* reported that it can be used as an early marker of CIN [16]. Laçin *et al.* reported that the groups with and without CIN did not differ with respect to serum NGAL levels [9]. In contrast, the current study showed that the 6th hour NGAL level was significantly higher in the group with CIN compared with the group without CIN. This difference between the current study and that of Laçin *et al.* may be explained by the observation that statistical significance could not be attained in the study by Laçin *et al.* since only two patients developed CIN.

The use of contrast agents has been steadily increasing with the widespread use of diagnostic and therapeutic interventional-radiological techniques. Increased use of contrast agents has led to a gradual increase in the rate of contrast-induced ARF [12, 13]. Patients with CIN suffer from prolonged hospital stays, increased hospital costs, increased rates of morbidity and mortality and increased rates of irreversible end-stage renal failure [20].

FIGURE 1. Receiver Operating Characteristic (ROC) curve of NGAL

The incidence of CIN has been reported between 1% and 9.2% [28, 30]. In this study, we found a CIN incidence of 16.2%. This higher incidence of CIN could be due to a number of factors, including a higher comorbidity rate and a mean population age greater than 50 years. It has been reported that age is a risk factor for the development of CIN [31]. It can be predicted that 11% of patients older than 70 years of age who undergo cardiac catheterization will develop CIN [20, 32]. In this study, the patients who developed CIN were older than the control group, probably resulting from an increased number of risk factors and declining renal function with aging.

Female gender is recognized as an independent predictor of CIN [32]. One study reported that male gender is a non-definitive risk factor for CIN development [33]. Laçin *et al.* failed to demonstrate any significant correlation between the occurrence of CIN and patient gender [9]. In our study, CIN was more prevalent in the female population, although this difference did not reach statistical significance. We are of the opinion that the female population is at greater risk owing to a greater number of risk factors.

Currently, CIN is diagnosed by serial measurements of serum creatinine levels. Despite the routine use of serum creatinine levels in clinical practice and clinical studies, the current trend is that serum creatinine levels alone may be insufficient for diagnosing ARF [34]. Blood urea level is an even less reliable indicator of renal function than creatinine level, since it is affected by many factors, including dietary

protein content, hepatic function and renal sodium avidity [35].

We observed that the urea and creatinine level were elevated at the 72nd hour in the CIN group. Urea and creatinine levels increased in both groups, but to a greater extent in the CIN group, consistent with the literature data.

The main limitation of the current study is the confinement of the study population solely to subjects with normal renal function; therefore, no conclusion can be drawn concerning patients with borderline renal function.

NGAL is a high cost marker; hence, social security institutions in Turkey do not include repayment for measurement of NGAL levels. Despite being costly to measure (at least for the time being), this cost is still negligible compared with that for renal replacement therapy (hemodialysis). Future studies exploring NGAL's role in patients with borderline renal function and specific age groups (pediatric, geriatric age groups, etc.) will hopefully pave the way for its utilization in daily practice.

NGAL levels can be determined at the 6th hour after a contrast-enhanced study, which would allow for earlier diagnosis of CIN and a timely start of appropriate therapy, particularly in “at risk” patient groups. NGAL levels may be re-determined at the 72nd hour, together with BUN and creatinine levels, to assess treatment efficacy. Our results should be confirmed by future prospective, randomized controlled studies.

Conclusion

By measuring NGAL levels at the 6th hour after a procedure, CIN can be detected and appropriate treatment initiated, thus preventing permanent renal damage.

Author contribution

MM and CK study design, EK and MF drafting article, AEK statistic, PD critical revision of article

Financial support

This study was supported by The Research Fund of Başkent University.

References

- Reddan D. Patients at high risk of adverse events from intravenous contrast media after computed tomography examination. *Eur J Radiol*. 2007;62:26-32.
- Solomon R. Contrast medium-induced acute renal failure. *Kidney Int*. 1998;53:230-42.
- McCullough PA, Adam A, Becker CR, Davidson C, Lameire N, Stacul F, et al. Epidemiology and prognostic implications of contrast-induced nephropathy. *Am J Med* 2006;98:5-13.
- Tublin ME, Murphy ME, Tessler FN. Current concepts in contrast media-induced nephropathy. *Am J Roent* 1998;171:933-9.
- Efrati S, Dishy V, Averbukh M, Blatt A, Krakover R, Weisgarten J, et al. The effect of N-acetylcysteine on renal function, nitric oxide, and oxidative stress after angiography. *Kidney Int* 2003;64:2182-7.
- Briquori C, Marenzi G. Contrast-induced nephropathy: Pharmacological prophylaxis. *Kidney Int*. 2006;69:30-8.
- Erley CM, Duda SH, Schlepckow S, Koehler J, Huppert PE, Strohmaier WL, et al. Adenosine antagonist theophylline prevents the reduction of glomerular filtration rate after contrast media application. *Kidney Int* 1994;45:1425-31.
- Lee YJ, Hu YY, Lin YS, Chang CT, Lin FY, Wong ML, et al. Urine neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute canine kidney injury. *BMC Vet Res* 2012;28:248.
- Lacin S, Kose M, Akpınar TM, Kayacan SM. The Role of NGAL in the Early Detection of Contrast Media Induced Nephropathy. *İc Hastalıkları Derg* 2013;20:91-5.
- Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993;268:10425-32.
- Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, Barasch J, Devarajan P. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2004;15:3073-82.
- Eisenberg RL, Bank WO, Hedgcock MW. Renal failure after major angiography can be avoided with hydration. *AJR* 1981;136:859-61.
- Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrington JT. Hospital-acquired renal insufficiency: a prospective study. *Am J Med* 1983;74:243-8.
- Padhy M, Kaushik S, Girish MP, Mohapatra S, Shah S, Koner BC. Serum neutrophil gelatinase associated lipocalin (NGAL) and cystatin C as early predictors of contrast-induced acute kidney injury in patients undergoing percutaneous coronary intervention. *Clin Chim Acta* 2014;435:48-52.
- Phillips JA, Holder DJ, Ennulat D, Gautier JC, Sauer JM, Yang Y, et al. Rat Urinary Osteopontin and Neutrophil gelatinase-associated lipocalin Improve Certainty of Detecting Drug-Induced Kidney Injury. *Toxicol Sci* 2016; pii: kfw038.
- Filiopoulos V, Biblaki D, Lazarou D, Chrisis D, Fatourou M, Sofia Lafoyianni S, Vlassopoulos D. Plasma neutrophil gelatinase-associated lipocalin (NGAL) as an early predictive marker of contrast-induced nephropathy in hospitalized patients undergoing computed tomography. *Clin Kidney J* 2013;6:578-83.
- Kim H, Hur M, Cruz DN, Moon HW, Yun YM. Plasma neutrophil gelatinase-associated lipocalin as a biomarker for acute kidney injury in critically ill patients with suspected sepsis. *Clin Biochem* 2013;46:1414-8.
- Humes HD, Hunt DA, White MD. Direct toxic effect of the radiocontrast agent diatrizoate on renal proximal tubule cells. *Am J Physiol* 1987;252:246-55.
- Makris K, Markou N, Evodia E, Dimopoulou E, Drakopoulos I, Ntetsika K, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. *Clin Chem Lab Med* 2009;47:79-82.
- Rihal CS, Textor SC, Grill DE, Berger PB, Ting HH, Best PJ, et al. Incidence and prognostic importance of acute renal failure after percutaneous coronary intervention. *Circulation* 2002;105:2259-64.
- Malyszko J, Bachorzewska-Gajewska H, Malyszko JS, Pawlak K, Dobrzycki S. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in hypertensive and normotensive patients with coronary artery disease. *Nephrology* 2008;13:153-6.
- Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, et al. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. *Arterioscler Thromb Vasc Biol* 2006;26:136-42.
- Malyszko J, Malyszko JS, Bachorzewska-Gajewska H, Poniatowski B, Dobrzycki S, Mysliwiec M. Neutrophil Gelatinase-Associated Lipocalin is a New and Sensitive Marker of Kidney Function in Chronic Kidney Disease Patients and Renal Allograft Recipients. *Transplantation Proc* 2009;41:158-1.
- Buget Mİ, Ozkilitci E, Kucukgergin C, Seckin S, Kucukay S, Yenigün Y, et al. Early Diagnosis in Acute Kidney Failure: Neutrophil Gelatinase Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), Interleukine-18 (IL-18), Cystatin C. *J Turkish Soc Intens Care* 2014;12:94-100.
- 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;54:1012-24.
- Kavalci C, Celikel E, Yilmaz MS, Yilmaz F, Arslan ED, Durdu T, Durukan P. The value of serum neutrophil gelatinase-associated lipocalin in determination of dialysis indication. *JPM* 2014;64:739-42.

27. Ozkan S, Durukan P, Kavalci C, Duman A, Sayhan MB, Salt O, İpekci A. Importance of neutrophil gelatinase-associated lipocalin in differential diagnosis of acute and chronic renal failure. *Iran Red Crescent Med J* 2014;16:e14133
28. Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Med* 2010;36:133-40.
29. Rudnick MR, Berns JS, Cohen RM, Goldfarb S. Contrast media-associated nephrotoxicity. *Curr Opin Nephrol Hypertens* 1996;5:127-33.
30. Barrett BJ, Carlisle EJ. Meta-analysis of the relative nephrotoxicity of high and low- osmolality iodinated contrast media. *Radiology* 1995;188:171-8.
31. Manske CL, Sprafka JM, Strony JT, Wang Y. Contrast nephropathy in azotemic diabetic patients undergoing coronary angiography. *Am J Med* 1990;89:615-20.
32. Iakovou I, Dangas G, Mehran R, Lansky AJ, Ashby DT, Fahy M, et al. Impact of gender on the incidence and outcome of contrast-induced nephropathy after percutaneous coronary intervention. *J Invasive Cardiol* 2003;15:18-22.
33. Toprak O, Cirit M. A Review of Prevention Strategies for Radiocontrast Nephropathy. *Turk Nephrol Dialy Trans J* 2004;13:111-6.
34. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol* 2005;16:3046-52.
35. Berg KJ, Jakobsen JA. Nephrotoxicity related to x-ray contrast media. *Adv X-ray Contrast* 1993;1:10-8.