

The Predictivity of Neutrophil Gelatinase Associated Lipocaline in the Development of Radiocontrast-Induced Nephropathy in the Intensive Care Unit Patients

Yoğun Bakım Hastalarında Radyokontrast Nefropati Gelişiminde Nötrofil Jelatinazla İlişkili Lipokalin Prediktivitesi

ABSTRACT

OBJECTIVE: The aim of this study is to investigate the predictive value of neutrophil gelatinase associated lipocaline (NGAL) levels in the development of radiocontrast-induced nephropathy (rin) in intensive care unit patients.

MATERIAL and METHODS: Forty patients (female: male was 23:17) with a mean age of 73.7± 9.7 yrs (range= 60-94 yrs) were included in the study. Iopromide 623mg/dl (ultravist 300) (iv) was administered at a dose of 1.5 ml/kg. NGAL measurements were performed with the ELISA method on serum samples (cut-off >25 ng/ml).

RESULTS: Eight patients were diagnosed as RIN. Five of these had 20% or more increases in NGAL levels. Cases who developed RIN had a statistically significant and direct correlation between the increases in NGAL and serum creatinine levels (p= 0.02). When the means of NGAL levels were compared before and six hours after the procedure, there was a significant increase after the procedure (p<0.001). For 32 patients who did not develop RIN, there was no statistically significant difference between NGAL levels before and six hours after the procedure (p>0.05).

CONCLUSION: When compared to creatinine, plasma NGAL levels help to establish the diagnosis of RIN at a much earlier stage.

KEY WORDS: Radiocontrast-induced nephropathy, NGAL, Intensive care unit patients

ÖZ

AMAÇ: Bu çalışmanın amacı, yoğun bakım hastaları arasından radyokontrast nefropatisi (RIN) gelişen hastalarda nötrofil jelatinazla ilişkili lipokalin (NGAL) prediktivitesini araştırmaktır.

GEREÇ ve YÖNTEMLER: Çalışmaya ortalama yaşı 73,7± 9,7 (aralık=60-94) olan kırk hasta (kadın:erkek oranı 23:17) alındı. Iopromide 623 mg/dl (ultravist 300) (iv) olarak 1,5 ml/kg dozda verildi. NGAL ölçümleri serum örneklerinde Elisa metodu ile yapıldı (cut-off >25 ng/ml).

BULGULAR: Sekiz hastada RIN tanısı konuldu. Bunlardan 5'inde NGAL seviyelerinde %20 veya daha fazla artış vardı. RIN gelişen olguların NGAL ile serum kreatinin düzeylerindeki artışlar arasında istatistiksel olarak anlamlı ve doğrudan ilişki vardı (p=0,02). NGAL düzeylerinin ortalamaları işlemden önce ve 6 saat sonra karşılaştırıldığı zaman işlemden sonra anlamlı bir yükselme vardı (p<0,001). RIN gelişmeyen 32 hastada, işlemden önce ve 6 saat sonraki NGAL düzeyleri arasında istatistiksel olarak anlamlı bir fark yoktu (p>0,05).

SONUÇ: Kreatininle mukayese edildiği zaman, plazma NGAL düzeyleri RIN tanısının daha erken bir evrede konulmasına yardımcı olabilir.

ANAHTAR SÖZCÜKLER: Radyokontrast nefropatisi, NGAL, Yoğun bakım hastaları

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INTRODUCTION

Radiocontrast-induced nephropathy (rin) is defined as an increase of 0.5 mg/dl or more in serum creatinine levels or as a 25% increase in basal serum creatinine levels within the 48 hours following the administration of contrast material (1). RIN has a prevalence of 3-5% in the general population while this increases to 40% in the presence of chronic renal failure and diabetes mellitus. In a patient who develops RIN, hospital mortality is 7.1 to 14.3% (2). RIN is seen more frequently due to the increase in the number of tests and interventional procedures requiring the use of contrast material.

To diagnose RIN, the increases in serum creatinine levels should be followed-up. In most of the patients, the serum creatinine levels start to increase within the first 24 to 48 hours following the administration of contrast material. The peak levels are attained within 3-5 days and the levels return to normal within 1-3 weeks. During recent years, new biomarkers have been studied for the early diagnosis of acute kidney injury (AKI) (3,4,5). Neutrophil gelatinase associated lipocalin (NGAL) is a member of the lipocalin superfamily with a molecular weight of 25 kd. It is a tubular protein in the kidneys and found in small amounts in the lungs, stomach and colon. Following ischemic and nephrotoxic damage, its expression increases in the distal tubule epithelium, while its reabsorption from the proximal tubulus is hindered. In AKI, it starts increasing in the plasma in 2 hours and in the urine in 4 hours and is a new marker for tubular inflammation (5,6,7).

In our study, we aimed at investigating the predictive value of plasma NGAL levels for the early detection of AKI following the use of radiocontrast material in moderate-risk patients according to Mehran risk scoring (1).

Subjects and methods

The study enrolled patients who were admitted to the intensive care unit with acute exacerbations of chronic obstructive pulmonary disease (COPD) or pneumonia-related acute respiratory distress between January and July 2011 after obtaining ethics approval. The patients were under mechanical ventilation, above the age of 60 years, had diagnoses of DM and serum creatinine levels of <1.3 mg/dl. They did not have any hypotension, decompensated heart failure, intraaortic balloon placement and had not been administered contrast material within the last week. These patients needed to have contrast computerized tomography (CT) for the diagnoses of their existing diseases and were categorized as moderate-risk according to Mehran risk scoring (1). Patients with spot urine microalbuminuria/ creatinine ratio > 300 mg/dl were excluded, which may explain the high levels of basal average creatinine clearance levels.

The patients received sodium chloride + 3 ampoules of 8.4 sodium bicarbonate and 600 mg n-acetyl cysteine for 6 hours at

doses of 3 ml/kg/hr 1 hour before the administration of contrast material and 1 ml/kg/hr after its administration. As for the choice of contrast material, for iv applications iopromide 623 mg/dl (ultravist 300) was used at a dose of 1.5 ml/kg. Basal creatinine levels (mg/dl) were measured before the procedure and further measurements were performed at 24 and 48 hours after the procedure. Creatinine clearance (ml/min) was calculated with Cockcroft-Gault. For plasma NGAL level measurements, blood samples were drawn under standard conditions before and 6 hours after the procedure. They were centrifuged immediately at 3000 rpm for 10 min. at room temperature. The aliquots of supernatants were stored at -20°C until the time of analysis. NGAL measurements were performed with elisa method (Biovendor human lipocalin-2/NGAL ELISA) on serum samples (cut-off >25 ng/ml). Serum samples were incubated in microplate wells pre-coated with polyclonal anti-human lipocalin-2 antibody. Biotin-labelled polyclonal anti-human lipocalin-2 antibody was added and incubated with captured lipocalin-2 for one hour. Then streptavidin-hrp conjugate was added and allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm.

Increases of 0.5 mg/dl or above in creatinine levels within 48 hours were taken as reference values for the diagnosis of RIN (1).

Statistical analyses:

Statistical analyses were performed with the statistical package for social sciences (SPSS) 15.0 program. In the analysis of the data, t-test and fisher's exact test were used for paired groups and r coefficient was obtained by performing correlation analysis. A p value of 0.05 was regarded as statistically significant.

RESULTS

Forty patients with a mean age of 73.7± 9.7 years (range= 60-94 years) were included in the study. The female to male ratio was 23/17. Mean Mehran RIN risk score was 13.4 ± 1.4 (11-15) corresponding to a moderate risk. Table I demonstrates the serum creatinine levels and creatinine clearance calculations of the patients before the administration of radiocontrast material and 24 and 48 hours thereafter. The means of creatinine and creatinine clearances before the procedure and at 24 hours after the procedure were not statistically significantly different from each other. When creatinine levels before contrast administration were compared with those at 48 hours after the procedure, there was a significant increase in creatinine levels (p=0,002). When the mean creatinine clearance before the procedure was compared to that 48 hours after the procedure, there was a significant decrease in the clearance (p=0.034).

Eight patients were diagnosed as RIN based on the ≥0.5 mg/dl increase in serum creatinine levels seen 48 hours after the

Table I: Serum creatinine and creatinine clearance levels of the patients before, and 24 and 48 hours after the administration of radiocontrast material.

(n= 40)	Before the procedure	At 24 hours	At 48 hours	P
Creatinine (mg/dl)	0.92±0.43	0.94±0.38	1.1±0.5	0.002*
Creatinine clearance (ml/min)	89.4±55.7	83.2±49.9	78.5±59.6	0.034**

*there was a significant increase in creatinine levels at 48 hours after contrast administration.

**there was a significant decrease in creatinine clearance at 48 hours after contrast administration.

Table II: NGAL levels of the patients before and 6 hours after the administration of radiocontrast material.

(n= 40)	Before the procedure	At 6 hours	P
NGAL (ng/ml)	246.1±16.8	260.9±16.3	<0.001

contrast administration procedure compared to those before the procedure. Five of the eight patients diagnosed a RIN had 20% or more increases in NGAL levels. Cases who developed RIN had a statistically significant and direct correlation between the increases in NGAL and serum creatinine levels ($p= 0.02$). When the means of NGAL levels were compared before and six hours after the procedure, there was a significant increase after the procedure ($p<0.001$) (Table II). For 32 patients who did not develop RIN, there was no statistically significant difference between NGAL levels before and six hours after the procedure ($p>0.05$).

Following the radiocontrast examination, all the patients had parallelisms between the increases in creatinine and NGAL levels. There was a significant correlation between the difference in serum creatinine levels before and 24 hours after the procedure and NGAL levels before and 6 hours after the procedure ($r=0,352$, $p=0.026$). There was also a significant correlation between the differences in creatinine levels before and 48 hours after the procedure and NGAL levels before and 6 hours after the procedure ($r=0,701$, $p<0.001$) (Table III). Among those patients who had 25% or more decreases in GFR after radiocontrast administration, 30.8% had increases of 20% or more in NGAL levels, whereas among those patients who had GFR decreases of less than 25%, this ratio was 3.7%. The

Table III: Cases were compared for creatinine difference and NGAL difference between pre- and after procedure.

	R	P
Creatinine difference between pre-procedure and after 24 hour	0.352	0.026
NGAL difference between pre-procedure and after 6 hour		
Creatinine difference between pre-procedure and after 48 hour	0.701	<0.001
NGAL difference between pre-procedure and after 6 hour		

Table IV: Cases that show decrease in GFR rates and 20% change in plazma NGAL values after radiocontrast.

Decrease in GFR	% increase in NGAL value				Total N (%)
	Below 20%		20% and above		
	N	%	N	%	
Decrease less than 25%	26	96.3	1	3.7	27
Decrease 25% and more	9	69.2	4	30.8	13
Total	35	87.5	5	12.5	40

difference between the two ratios was statistically significant ($p=0.031$) (Table IV).

Five of the eight patients who developed RIN after the radiocontrast examination (serum creatinine increase of 0.5 mg/dl and above) had NGAL level increases of 20% or more. Among the 32 patients who did not develop RIN (serum creatinine increase of less than 0.5 mg/dl), only 1 patient had a NGAL level increase of more than 20%. Following the administration of contrast material, 20% or more increase in NGAL levels at 6 hours compared to basal levels had a sensitivity of 62.5% specificity of 96.8% for the development of nephropathy.

DISCUSSION

Patients diagnosed as RIN did not show a significant difference for creatinine and creatinine clearance levels at 24 hours ($p>0.05$). The difference between these parameters was significant at 48 hours and was in correlation with the findings in the literature. This situation shows us that following-up the changes in the levels of creatinine and creatinine clearance within the first 24 hours will not provide any guidance for diagnosing the development of RIN.

Although patients in this study had acute distress syndrome with mechanic ventilation, diabetic and over 60 years old, their

creatinine levels were <1.3 mg/dl without proteinuria. This was consistent with Mehran moderate-risk scoring.

There was no acute kidney injury (AKI) in our patients since we had used previous definition of AKI. Currently AKI is defined as any of the following: increase in SCR by ≥ 0.3 mg/dl within 48 hours; increase in SCR to ≥ 1.5 times baseline; or urine volume <0.5 ml/kg/h for 6 hours. According to these criteria; it would be possible to include more AKI patients in our study (8).

Basal plasma NGAL levels of the cases were 246 ± 116.8 ng/ml. There was no fixed reference level mentioned in the literature, in certain studies reference levels were stated as 50-100 ng/ml for patients who did not have any renal problems and as 200 ng/ml for those with AKI (9,10). The patients recruited to our study were critical patients and due to their primary reasons of hospitalization, their mean basal NGAL levels were 200 ng/ml or above. In the previously conducted studies, there was no fixed reference NGAL level mentioned. That is why instead of assigning a certain numerical NGAL level for our study, as a reference we employed having 20% or more of an increase in NGAL levels at six hours after the procedure.

Compared to the means of NGAL levels before the procedure, the mean NGAL levels 6 hours after the procedure were significantly higher ($p < 0.001$). In our study, the proportional increase in NGAL levels was seen to have been in correlation with the clinical course. Five (62.5%) of the eight (20%) patients diagnosed as RIN had increases of 20% or above in NGAL levels. The correlation between the increases in the levels of these two parameters was statistically significant and directly proportional ($p = 0.02$). After the administration of contrast material, having 20% or more of an increase in NGAL levels at 6 hours compared to basal levels had a specificity of 96.8% and a sensitivity of 62.5% for the development of nephropathy.

When compared to creatinine, plasma NGAL levels help to establish the diagnosis of RIN at a much earlier stage. Diagnosing RIN at an early stage will make it possible to employ prophylactic measures at an earlier phase especially for critical patients and will help reduce mortality and morbidity.

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