



# Study of neutrophil gelatinase-associated lipocalin in patients with cardiovascular shock

Aiyoub Pezeshgi<sup>1,2</sup>, Samad Ghodrati<sup>2</sup>, Mina Kiafar<sup>2</sup>, Kurosh Kamali<sup>3</sup>, Masoud Asadi-Khiavi<sup>4,5\*</sup>

<sup>1</sup>Zanjan Metabolic Disease Research Center, Zanjan University of Medical Sciences, Zanjan, Iran

<sup>2</sup>Department of Internal Medicine, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

<sup>3</sup>Department of Epidemiology, Zanjan University of Medical Sciences, Zanjan, Iran

<sup>4</sup>Zanjan Applied Pharmacology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran

<sup>5</sup>Department of Pharmacotherapy, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran

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## ABSTRACT

**Introduction:** Acute kidney injury (AKI) makes a reversible accumulation of nitrogen products. This waste product is partly determined by serum creatinine level but it is not reliable during hypotension. However, neutrophil gelatinase-associated lipocalin (NGAL), as a new biomarker, shows an obvious increase even at hypotensive status.

**Objectives:** The presented study was designed to evaluate NGAL as a right biomarker for AKI early diagnosis and consequent appropriate therapies.

**Patients and Methods:** In this study, 25 healthy individuals and 47 cases out of 60 primarily admitted patients with low blood pressure were evaluated for NGAL level using blood samplings, health documents as well as analysis of questionnaires data. The group's sizes were determined based on AKI and hypotension risk rates. Exclusion and inclusion criteria were firmly considered to avoid major confounding factors.

**Results:** AKI was found in 20 cases out of 47 hypotensive patients. NGAL levels were about  $243.35 \pm 105.74$  ng/dL (Mean  $\pm$  SD) in AKI and  $192.32 \pm 64.31$  ng/dL (Mean  $\pm$  SD) in non-AKI hypotensive patients that showed a significant difference ( $P=0.037$ ) at the first 6 hours. There was no significant difference between hospitalization duration and NGAL level ( $P=0.616$ ).

**Conclusion:** NGAL is important diagnostic protein in the early stages of AKI while there was no creatinine increasing. On the other hand, NGAL level during early 6 hours of hypotension introduces it as an indicative biomarker of AKI based on provided literature and our presented results.

### Implication for health policy/practice/research/medical education:

In the presented study, the diagnostic advantage of neutrophil gelatinase-associated lipocalin (NGAL) was illustrated in AKI. On the other hand, it is concluded that the NGAL is very useful biomarker particularly at the early stages of low blood pressure status in comparison with the routine serum creatinine.

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## Introduction

Acute renal injury refers to a rapid decline in kidney excretory and filtration function. It leads to accumulation of waste products such as creatinine as well as following potassium and phosphate increase (1). More than 30% of acute kidney injury (AKI) patients show serum creatinine increase by the rate of 0.3 to 0.4 mg/dL. Unfortunately, up to 70% of mentioned cases illustrate the high rate of mortality in comparison with normal creatinine one (2).

The AKI odds increase by 3% per 1 mm Hg decrease in mean arterial pressure (MAP) less than 80 mm Hg. Additionally, the risk of AKI enhances two times per hour every 10-mm Hg MAP drop off (3). Several biomarkers were introduced in the case of AKI. One of these markers is serum creatinine that partly indicates nitrogen waste products level but it is not reliable during hypotension. Other biomarker is neutrophil gelatinase-associated lipocalin (NGAL) that showed some advantages rather

\*Corresponding author: Masoud Asadi-Khiavi, Email: makhavi@gmail.com

than creatinine. The urinary concentration of NGAL quickly increases during early AKI. Several studies demonstrated that plasma and urine level of NGAL increase during inflammatory processes as well (4). NGAL is discovered in three molecular types; 25-kDa monomer, 45-kDa disulfide-linked homodimer and 135-kDa heterodimer that covalently conjugated with gelatinase [matrix metalloproteinase (MMP)-9] (4-6). These molecules are synthesized by bone marrow and stored within neutrophil granules. Moreover, NGAL mRNA was detected in several organs such as colon, trachea, lung and kidney epithelium (7). Because of NGAL rapid elevation in AKI, it was considered as one of the promising markers for prediction of AKI in Iranian people who need to get appropriate medical services at the right time and as soon as possible.

### Objectives

Presented study was designed to evaluate NGAL as a suitable biomarker for early diagnosis of AKI and consequent proper therapies.

### Patients and Methods

#### Patients

A total of 25 healthy volunteers were selected as a group for determining the basal level of NGAL. Additionally, 60 hospitalized patients who had blood pressure drop less than 80 mm Hg were primarily considered for comparing accordingly. Inclusion criteria for cases were MAP less than 80 mm Hg as well as glomerular filtration rate (GFR) lower than 30 mL/min (based on Cockcroft-Gault equation). Exclusion criteria for cases were about patients who used nephrotoxic agents like antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin receptor blockers (ARBs) and angiotensin-converting-enzyme inhibitor (ACE inhibitor) drugs during the week before hypotension attack or patients who administered them during the first 3 days of the hypotension attack. Increased serum bilirubin and death were other exclusion criteria.

#### Study design

RIFLE (Risk, Injury, Failure, Loss and ESRD; end stage renal disease) criteria were considered for AKI defining and data gathering. Mentioned 25 healthy volunteers and 60 hospitalized patients were enrolled in the presented survey as a cohort study and blood sampling were done for each group. Creatinine (Cr) and NGAL serum levels were primarily determined at the time of admission and second sampling was performed during first six hours for both groups (during first six hours of blood pressure drop in the case of hospitalized one). Serum creatinine level was also assessed for three consecutive days in the case of hospitalized patients. Serum NGAL level was compared to serum Cr level as a biomarker for AKI. Demographic information such as age, gender, concomitant diseases such as hypertension, diabetes and coronary artery

disease was registered in the questionnaires. Subsequently, blood samples (5 mL) were obtained in the pattern that mentioned previously. The serum level of NGAL was detected by fluorescence-detected immunoassay (ELISA) with the triage meter using Biotech Cristal Day kit.

#### Ethical issues

Study related information was delivered to patients and informed consent was provided before using questionnaires. Other information and patients follow up (statues including kidney injury recurrence, potential kidney injury severity, need for dialysis, etc.) were obtained using patients' records and declaration. The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Zanjan University of Medical Sciences approved this study (ethical code number# 1-3).

#### Statistical analysis

Acquired data were analyzed via paired *t* test and analysis of variance (ANOVA) following by Bonferroni post-hoc test using SPSS software version 18. The non-parametric tests were used in the case of values without normal distribution. The level less than 0.05 was considered as significant for *P* value.

### Results

#### Enrolled cases

As it is mentioned already, a total number of 25 healthy volunteers as well as 60 hospitalized patients were enrolled in study. According to mentioned exclusion criteria, 47 out of 60 patients were evaluated for NGAL level. The mean  $\pm$  SD age for healthy volunteers group (12 females and 13 males) was  $29.7 \pm 6.8$  years old. This value was about  $59.6 \pm 20.9$  years in case of patients group (19 females and 28 males).

#### NGAL level values

Serum creatinine and NGAL mean ( $\pm$ SD) level in the healthy volunteers group were  $0.9 \pm 0.2$  mg/dL and  $246.5 \pm 84.4$  ng/mL respectively. There was no significant difference between mean values of age and NGAL level in healthy volunteers group, but serum creatinine mean level showed a significant difference between two genders ( $P=0.04$ ). In the first day, creatinine mean level was  $0.9 \pm 0.1$  mg/dL and was  $1.3 \pm 0.9$  mg/dL in the third day that showed an elevated value in comparison with first day. Serum NGAL mean level in this group was about  $214 \pm 87.2$  ng/dL. The average length of hospitalization was  $12.3 \pm 16.3$  days. Unfortunately, thirteen patients expired during hospitalization and no need to use hemodialysis during the study. Creatinine levels of 14 patients showed enhancement up to AKI range. Additionally, 16 patients showed a decline in urine output and 20 patients suffered from AKI based on RIFLE criteria. Mean NGAL level of patients who diagnosed as hypotensive AKI (20 cases) and hypotensive cases without AKI (27 patients) were  $243.3 \pm 105.7$  ng/mL and  $192.3 \pm 64.1$  ng/mL respectively that

showed a significant difference ( $P=0.03$ ) (Bonferroni post hoc test). There was no significant difference between mean NGAL level of healthy volunteers and AKI as well as non-AKI hypotensive patients ( $P=0.89$ ). Mean creatinine level of the blood samples in patients group showed significant differences using t-test between second day ( $P=0.003$ ) and third day ( $P=0.001$ ) measurements. Additionally, statistical difference between average length of hospitalization period and AKI occurrence was not significant based on mentioned t-test ( $P=0.236$ ). Chi-square test showed no significant relationship between average length of hospitalization period and mean NGAL level ( $P=0.333$ ). There was a significant difference between the occurrence of AKI and the RIFLE criteria regarding serum creatinine among first ( $P=0.04$ ), second ( $P<0.001$ ) and third days ( $P<0.001$ ) using t-test. However, such significant differences were not showed about the mean NGAL level ( $P=0.062$ ). There were significant differences at the second day ( $P=0.042$ ) and third day ( $P=0.04$ ) between creatinine and mean NGAL level ( $P=0.022$ ) in the cases of AKI occurrence and the urinary output except for first day creatinine that showed no significant differences ( $P=0.972$ ).

## Discussion

Mean creatinine level differences were statistically significant between two genders ( $P=0.04$ ). This finding is similar to other studies and probably it was about genders with different muscular mass (8). There were significant differences ( $P=0.037$ ) between mean NGAL levels of hypotensive patients with or without AKI particularly at the early stages of AKI that serum creatinine shows no change. It means that NGAL illustrates a unique property to be considered as a bio-indicator during hypotension as the main etiology of AKI. It was confirmed that AKI occurrence in sepsis was accompanied by high level of NGAL at the admission time (9). It was also showed that critically ill children who affected by SIRS (systemic inflammatory response syndrome) and suspected to severe sepsis have had a significant relation between AKI and NGAL's serum level particularly in first 24 hours of hospitalization (10). There was no significant difference between NGAL level and patient's mortality ( $P=0.381$ ) in our study. In one study, elevated NGAL level because of malignancy surgery as well as association with post-operation AKI was showed no significant differences between mortality rate and NGAL levels that were as the same of our finding (11). These results are the same as our previous released document in the case of kidney transplantation (12). In other study that handled on 45 patients with cardiogenic shock, it is showed that NGAL level increase at second they can be considered as a mortality predictor for patients with complicated AKI (13). Our findings showed no statically significant differences between serum creatinine and NGAL levels ( $P=0.062$ ) based on RIFLE criteria. According to RIFLE criteria, urine output values showed significant differences in the

case of serum creatinine level at the second ( $P=0.042$ ) and third ( $P=0.04$ ) days as well as urine NGAL level ( $P=0.022$ ) based on our study. This fact was shown in a study on 83 hospitalized patients who admitted in ICU. They measured urine output, NGAL and serum creatinine levels at 24 and 48 hours after hospitalization. They showed a negative relationship between urine NGAL level in severe AKI patients and urine output at the first and second days. However, a direct relation between urine NGAL and serum creatinine at the first day was observed (14). It is illustrated that plasma levels of NGAL at the admission time in post-cardiac surgery ICU is related to average length of hospitalization period in ICU (15). It is against our finding that showed no statically significant difference between NGAL level and average length of hospitalization period. It probably shows less power of our study to judge about relationship between NGAL level and outcomes of AKI. However, variation in outcomes are related to the complexity of subcellular biochemistry and need to understand interactions between genes and use genetic regulatory networks modeling for elucidating molecular bases of mentioned multiple consequences (16).

## Conclusion

The NGAL level increase within the first six hours of hypotension can be considered as an indicator for early AKI during their hospitalization period. Additionally, patients at risk for AKI whose creatinine level has not elevated yet can be detected using NGAL level.

## Limitations of study

According to the variety of NGAL level measurements and limited size of healthy volunteers group, further studies with the large size of healthy volunteers is essential.

## Authors' contribution

AP; scientific writing, principal co-author, coordinator. SG; second co-author, help in scientific writing. MK; patients detecting. KK; statistical analysis. MAK; principal author, corresponding author.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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