The Role of Serum Neutrophil Gelatinase-associated Lipocalin in the Early Diagnosis of Nephropathy in Patients with Acute Alcohol Poisoning

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Abstract

AIM: In our study, we assessed the possibility of using the serum neutrophil gelatinase-associated lipocalin (NGAL) for the early detection of kidney damage in patients with acute alcohol poisoning (AAP).

METHODS: The study included 89 patients and 30 healthy donors. All participants in the study were mostly represented by men (90%) aged between 20 and 40 years. The influence of alcohol poisoning severity was also taken into account in the study. The Human NGAL ELISA Kit was used for the quantitative detection of serum NGAL. We also evaluated the main laboratory indicators of kidney functions, including eGFR (calculated according to serum creatinine).

RESULTS: We did not find a correlation between blood alcohol concentration and serum NGAL level; also, alcohol poisoning severity did not affect the NGAL values. The results of our study showed the possibility of using the serum NGAL in patients with AAP to detect the preclinical stage of reduced renal function, until the moment when it can be diagnosed with using only serum creatinine.

CONCLUSION: We propose to consider an increase in eGFR together with an increase in serum NGAL in this group of patients as a stage, preceding nephropathy, even in the absence of clinical and laboratory signs of impaired renal function.

Introduction

Alcohol use has been identified as a major risk factor for disease burden and premature mortality and as a global significant problem [1]. Alcohol poisoning is constantly recorded in many countries around the world. Kazakhstan ranks 34th place from 188 countries in the world (10.96 L of alcohol per capita) and first among the countries of Central Asia [2]. The overall mortality from alcohol poisoning in this region averages 80% [3]. The number of alcohol poisoning in Kazakhstan in 2013 was 86.6 per 100,000 thousand population, and the number of alcohol poisoning deaths was 5.6/100,000 population [2]. Among the acute exogenous poisonings, alcohol poisoning is 34.67% and second only to drug poisoning – 44.73% [4]. Drinking too much in a short period of time may result in a coma and death. One of the pathological syndromes in acute alcohol poisoning (AAP) is toxic nephropathy – defeat of the glomerular apparatus and kidney tissue [5]. The changes are due to the direct toxic effect of high concentrations of ethanol and acetaldehyde on the renal tissue, as well as impaired microcirculation.

The course of nephropathy is prolonged in time, irreversible, and progressive. In the preclinical stage – there is an accumulation of structural changes in the kidney, which could be revealed, until sometime, only by puncture biopsy. The first clinical-stage is characterized by proteinuria, in some cases passing into the stage of nephrotic syndrome and, as the outcome, the stage of chronic renal failure (CRF) [6]. The term “preclinical kidney disease” has become widely known since 2006 [7], [8]. It began to be used for persons with normal glomerular filtration rate (eGFR, according to creatinine level), but with elevated serum cystatin C level. It was proposed to use cystatin C detection to identify patients at high risk of chronic kidney disease (CKD) [7], [9]. For the early diagnosis of CKD and detection of renal damage, it has also been proposed to use neutrophil gelatinase-associated lipocalin (NGAL) – specific protein, the level of which in blood plasma or urine gradually increases during the development of renal dysfunctions [10], [11]. NGAL has been shown to be effective in the early diagnosis of acute kidney injury (AKI) in various clinical settings [12], [13], [14]. The presence of such markers opens up the possibility of detection the "preclinical
kidney disease,” as well as timely treatment and prevention of the disease progression to the CRF (corresponding to CKD 3–5 stages). At present, the limitations of serum creatinine for the early detection and accurate assessment of kidney damage are widely known because serum creatinine rises only when the function of about 50% of nephrons is lost [15], [16], [17].

The toxicological departments in our country most often use the classification of toxic nephropathy and its diagnostic criteria proposed by Luzhnikov and Kostomarov back in 1989 [18]. At the same time, despite the subsequent appearance of improved classifications, the decrease in eGFR, progressive with increasing the nephropathy severity remains a common diagnostic criterion for them. However, as mentioned before, the experience of recent years, in particular with diabetic nephropathy, shows the “insensitivity” of serum creatinine to the early stages of kidney damage and its inability to detect the preclinical stage. Late diagnosis significantly reduces the effectiveness of treatment and, as known, AKI is a strong risk factor for CKD.

Purpose of the study: The purpose of this study was to assess the possibility of using the serum NGAL for the early detection of kidney damage in patients with AAP. In addition, the impact of the alcohol poisoning severity and eGFR (calculated according to serum creatinine) on the level of NGAL was studied.

Methods

This prospective cross-sectional study was conducted on the basis of the biochemical laboratory of Karaganda Medical University together with the toxicological department of the Regional Medical Center (from January 2018 to May 2019). The diagnosis of alcohol poisoning and determination of the severity of intoxication were carried out according to the protocol “The toxic effect of alcohol (adults and children)” recommended by the Expert Council of the Ministry of Health and Social Development of the Republic of Kazakhstan (30.10.2015) with the obligatory determination of blood alcohol concentration (BAC). The diagnosis confirmation was based on a thorough medical history, an objective examination, laboratory tests, and determination of BAC. In addition to objective data and laboratory tests (AST/ALT ratio and serum GGT activity), the CAGE questionnaire was used to confirm AAP and exclude chronic alcohol intoxication.

The study included 89 patients with AAP and 30 healthy donors (control group). In the study, the influence of alcohol poisoning severity (moderate degree, n = 42; severe degree, n = 47) was also taken into account. All participants in the study were mostly represented by men (90%) aged between 20 and 40 years. Exclusion criteria from the study were as follows: Chronic alcohol intoxication, alcoholic or viral hepatitis, the presence of acute infectious and inflammatory processes of other organs during the study period, as well as acute or chronic pyelonephritis of infectious etiology, acute or chronic glomerulonephritis, diabetes and/or diabetic nephropathy, and obesity. In addition, persons younger than 18 years or older than 40 years were excluded from the study. Blood sampling was carried out early in the morning on the 2nd day of hospitalization since it is believed that this time is enough for a response of serum creatinine in the case of AKI [19]. Blood was stabilized by heparin. All blood tests were conducted within 2 h after the blood collection. The Human NGAL ELISA Kit (Affymetrix eBioscience, Austria) was used in the study for the quantitative detection of serum NGAL, the unit of measure was pg/ml. The main laboratory indicators of kidney functions were also evaluated. eGFR was calculated according to serum creatinine, using the CKD-EPI formula (unit in ml/min/1.73 m²). Patients’ BAC averaged 2.2 permille (‰, BAC by mass) with a total range of 0.6–4.7 ‰.

Ethical issues

The study was approved by the Ethics Committee of Karaganda Medical University and was conducted in accordance with the Helsinki Declaration. Informed consent from patients and healthy subjects for participation was obtained before the study. During the presence of patients in the toxicological department, all of them received standard therapy, corresponding to the poisoning severity and developed complications.

Statistical analysis

The program Statistica for Windows, version 12, was used to analyze the received data. One-way ANOVA for independent variables was used [20] to determine significant differences between the groups. The choice of this statistical method was due to the normal distribution of data (Shapiro–Wilk normality test, p > 0.05) and homogeneity of variances (Levene’s test, p = 0.591) [21]. The differences were considered reliable at significance level p < 0.05. To identify pairs of samples, differing from each other in means, we used the post hoc Tukey test. In addition, Pearson’s correlation analysis was used to estimate correlations between the studied parameters.

Results

To study the impact of eGFR on the serum NGAL concentration in patients with AAP, we ranked conditionally eGFR indexes into three groups: From 90 to 120 ml/min/1.73 m² – “normal” eGFR, above
A slight increase in the level of NGAL in patients with AAP in the group with “normal” eGFR without clinical and laboratory signs of impaired renal function is possible, in our opinion, due to the direct effect of ethanol on the liver. In addition, all patients had leukocytosis with a high percentage of segmented neutrophils. It was previously reported that the liver and neutrophils are sources of NGAL in the blood [12]. In our study, we did not find a correlation between BAC and serum NGAL level; also, alcohol poisoning severity did not affect the NGAL values. The correlation between eGFR and BAC is due to the effect of ethanol. The mechanisms of eGFR increasing in the case of AAP have been described in detail and are associated mainly with hypertonic dehydration and an increase in the osmolarity of blood plasma [22]. It deserves attention, that the same mechanism, due to hyperglycemia, underlies the increase in eGFR in the case of diabetic nephropathy.

The results of our study showed that a multiple increases in the level of serum NGAL not only in patients with nephropathy and reduced eGFR but also in patients with “increased” eGFR even in the absence of clinical and laboratory signs of impaired renal function, as well as in patients with “normal” or “increased” eGFR and minor shifts in urine analysis. A control group (individuals without clinical and laboratory signs of impaired renal function and without signs of AAP) was used as a comparison group.

The level of serum NGAL in the analyzed groups is presented in Table 1. Thus, in Group I with “normal” eGFR without clinical and laboratory signs of impaired renal function, the level of NGAL increased slightly, but without a statistically significant difference with the control group. In Group II with “increased” eGFR, the level of NGAL increased significantly on average by 4.5 times compared with the control group (p = 0.043). In Groups III and IV, a significant increase in the serum NGAL concentration was also detected (p = 0.026 and p < 0.001, respectively). The maximum values, reaching 1100 pg/ml, were found in Group IV patients with toxic nephropathy caused by AAP. The level of NGAL in this group exceeded both the values of the control group and Group I with “normal” eGFR without clinical and laboratory signs of impaired renal function (Table 1).

Alcohol poisoning severity, according to our data, did not have a statistically significant effect on the serum NGAL concentration (F = 0.81, p = 0.37). Hence, the level of NGAL was much higher than the values of the control group both in moderate degree (m = 527.02; CI 95%: 334.06–719.98) and severe degree of alcohol poisoning (m = 425.25; CI 95%: 285.31–565.19), (p < 0.005). In addition, a correlation analysis showed that the NGAL level did not correlate with the BAC, while the eGFR correlated with BAC (r = 0.26, p < 0.05) and the higher BAC, the higher eGFR.

### Table 1: Serum NGAL concentration in the analyzed groups

| Analyzed groups | Mean (pg/ml) | <95% CI | >95% CI |
|-----------------|-------------|---------|---------|
| Control group   | 102.57      | 42.23   | 162.91  |
| Group I         | 219.64      | 111.83  | 396.48  |
| Group II        | 459.15*     | 290.59* | 627.72* |
| Group III       | 561.52*     | 216.48* | 930.56* |
| Group IV        | 601.02*     | 353.58* | 848.45* |

*Reliability of differences with the control group, p>0.05. 1Reliability of differences with the Group I, p=0.007. CI: Confidence interval.
an increase in serum NGAL in this group of patients as a stage, preceding nephropathy, even in the absence of clinical and laboratory signs of impaired renal function. Obviously, the changes in the kidneys are reversible exactly at the preclinical stage in the case of elimination of etiological factor and appropriate therapy. While in diagnosed nephropathy, even though the etiological factor is eliminated, the renal function worsens progressively, and the stage of CRF develops as an outcome [6], [24]. However, the main point why and due to what mechanisms the changes in the kidneys that have arisen initially continue to progress inevitably even with the elimination of etiological factor has not yet found an exhaustive explanation.

**Conclusion**

Our study showed the possibility of using the serum NGAL in patients with AAP to detect the preclinical stage of reduced renal function, until the moment when it can be diagnosed with using only serum creatinine. We propose to consider an increase in eGFR together with an increase in serum NGAL in this group of patients as a stage, preceding nephropathy, even in the absence of clinical and laboratory signs of impaired renal function.

**References**

1. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Lonning P, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2224-60. https://doi.org/10.1016/S0140-6736(12)61728-6

2. Nashkenova A, Zhamalieva D, Zholasuybeykova A. Alcoholism in Kazakhstan (statistical data analysis). Vestnik KazNPU. 2015;2:201-5. Available from: https://www.cyberleninka.ru/article/n/rasprostranennost-alkogolizma-v-kazakhstane-obzor-i-interpretatsiya-statsisticheskikh-dannyh. [Last accessed on 2019 Jul 10].

3. Eskalieva AT, Kisina MS, Kuderina LT. Drug Addiction Treatment to the Population of the Republic of Kazakhstan. Statistical Book for 2013-2014. Republican Scientific and Practical Center of Medical and Social Problems of Drug Addiction. Geneva, Switzerland: UNAIDS; 2015. p. 25.

4. Kraeva Yu V, Brusin KM, Kondrashov DL, Sentsov VG, Hovda KE. Pre-hospital and hospital acute poisoning pattern study. Biomed J Med 2013;14:750-61.

5. Babanin AA, Belovitsky OV, Skrebkova OI, Sherbakova VM. Current understanding about the damages of the kidneys in alcoholic intoxication (literature review). Crimea J Exp Clin Med 2011;1:150-4.

6. Vereschagina TD. Classification of secondary nephropathies and chronic kidney disease (CKD). Sibir Med Rev. 2012;1(73):100-3. Available from: https://www.cyberleninka.ru/article/n/k-voprosu-o-klassifikatsii-vtorichnyh-nefropatii-i-chronicheskoy-bolezni-pochech-hip. [Last accessed on 2019 Jul 01].

7. Shlipak MG, Katz R, Sarnak MJ, Fried LF, Newman AB, Stehman-Breen C, et al. Cystatin C and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease. Ann Intern Med. 2006;145(4):237-46. https://doi.org/10.7326/0003-4819-145-4-200608150-00003
PMid:16908914

8. Vel’kov VV, Cystatin C and NGAL the markers of preclinical renal dysfunction and subclinical acute kidney injury. Lab Sluzhba. 2015;4(2):38-43. Available from: https://www.mediasphera.ru/issues/laboratornaya-sluzhba/2015/2/032305-2198201526. [Last accessed on 2019 Jul 05]. https://doi.org/10.17116/labs20154238-43

9. Peralta CA, Katz R, Sarnak MJ, Ix J, Fried LF, De Boer I, et al. Cystatin C identifies chronic kidney disease patients at higher risk for complications. J Am Soc Nephrol. 2011;22(1):147-55. https://doi.org/10.1681/asn.2010050483
PMid:2164029

10. Woo KS, Choi JI, Kim BR, Kim JE, An WS, Han JY. Urinary neutrophil gelatinase-associated lipocalin levels in comparison with glomerular filtration rate for evaluation of renal function in patients with diabetic chronic kidney disease. Diabetes Metab J. 2012;36(4):307-13. https://doi.org/10.4093/dmj.2012.36.4.307
PMid:22950063

11. Markova TN, Sadovskaya VV, Bespyatova MY. Modern methods of diagnosing chronic kidney disease in patients with diabetes mellitus. Diabetes Mellitus. 2017;20(6):454-60. Available from: https://www.endojournals.ru/index.php/dia/article/view/9268/0. [Last accessed on 2019 Jul 05]. https://doi.org/10.14341/dm0926

12. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: A critical evaluation of current status. Ann Clin Biochem. 2014;51(Part 3):335-51. https://doi.org/10.1177/0004563214521795
PMid:24518531

13. Haase M, Bellomo R, Devarajan P, Ma Q, Bennett MR. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. Ann Thorac Surg 2009;88(1):124-30. https://doi.org/10.1016/j.athoracsur.2009.04.023
PMid:19559209

14. Hawkins R. New biomarkers of acute kidney injury and the cardio-renal syndrome. Korean J Lab Med. 2011;31(2):72-80. https://doi.org/10.3343/kjlm.2011.31.2.72
PMid:21474979

15. Doi K, Yuen PS, Eiser C, Hu X, Leeahavanichkul A, Schernmann J, et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. J Am Soc Nephrol. 2009;20(6):1217-21. https://doi.org/10.1681/asn.2008060617
PMid:19389851

16. Mehta RL. Biomarker explorations in acute kidney injury: The journey continues. Kidney Int. 2011;80(4):335-51. https://doi.org/10.1111/j.1523-1755.2011.01585.x
PMid:21799504

17. Iau P, Saganova ES, Smirnov AV. Biomarkers in the diagnosis of acute kidney injury. Nephrology (Saint-Petersburg). 2014;18(4):335-51. https://doi.org/10.1111/nph.12066
PMid:24518531

18. Luzhnikov EA, Kostomarov LA. Acute Poisoning: A Guide for Doctors. Moscow: Medicine; 1989.

19. Ostermann M, Joannidis M. Acute kidney injury 2016: Diagnosis and diagnostic workup. Crit Care. 2016;20(1):299. https://doi.org/10.1186/s13054-016-1479-z
PMid:27670788
20. McDonald JH. Handbook of Biological Statistics. 3rd ed. Baltimore, Maryland, USA: Sparky House Publishing; 2014. p. 146-8.

21. Grjibovsky AM. Analysis of three and more independent groups of quantitative data. Hum Ecol J. 2008; (3):50-8. Available from: https://www.cyberleninka.ru/article/n/analiz-treh-i-bolee-nezavisimyh-grupp-kolichestvennyh-dannyh. [Last accessed on 2019 Jul 02].

22. Kursov SV, Mikhnevich KG, Krivobok VI. Acute poisoning with ethanol. Med Emerg States. 2012; 7-8(46-47):22-35. Available from: https://www.cyberleninka.ru/article/n/ostroe-otravlenie-etanolom. [Last accessed on 2019 Jul 01].

23. Zelveian PH, Dheryan LG. Glomerular filtration rate as a marker of kidney damage in patients with arterial hypertension. Eur Heart J. 2014; (2):44-8. Available from: https://www.cyberleninka.ru/article/n/skorost-klubochkovoy-filtratsii-kak-pokazatel-porazheniya-pochek-u-bolnyh-s-arterialnoy-gipertenziei. [Last Accessed on 2019 Jul 02].

24. Lopez-Novoa JM, Rodriguez-Pena AB, Ortiz A, Martinez-Salgado C, Lopez Hernandez FJ. Etiopathology of chronic tubular, glomerular and renovascular nephropathies: Clinical implications. J Transl Med. 2011;9:13. https://doi.org/10.1186/1479-5876-9-13 PMid:21251296