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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 m^2). 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 m^2 – "normal" eGFR, above

120 ml/min/1.73 m^2 – "increased" eGFR (from 121 to 140), and <90 ml/min/1.73 m^2 – "reduced" eGFR (from 50 to 89). The effect of gender was excluded because the study was dominated by men. We found that eGFR influenced the level of serum NGAL ($F = 4.29$, $p = 0.01$) and increased NGAL was observed both at "reduced" and "increased" eGFR relative to the control group values ($p = 0.035$ and $p = 0.012$, respectively).

Next, to analyze the effect of an increase in serum NGAL concentration, we added the results of laboratory tests of kidney function to the study. We divided the patients with AAP into the following groups: Group I (n = 25) – with "normal" eGFR without clinical and laboratory signs of impaired renal function; Group II (n = 20) – with "increased" eGFR without clinical and laboratory signs of impaired renal function; and Group III (n = 17) – with "normal" or "increased" eGFR and minor shifts in urine analysis: Moderate leukocyturia (from 4 to 14 cells in the field of view) and minor proteinuria (0.03–0.1 g/l). And finally, Group IV (n = 27) – patients with toxic nephropathy caused by AAP (it corresponds to the clinical picture of AKI), confirmed by proteinuria, hematuria, leukocyturia, cylindruria, increased serum creatinine, as well as reduced eGFR and daily diuresis. 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	−95% CI	+95% CI
Control group	102.57	42.23	162.91
Group I	219.64	111.83	327.46
Group II	459.15*	290.59*	627.72*
Group III	561.52*	219.48*	903.56*
Group IV	601.02*[&]	353.58*[&]	848.45*[&]

*Reliability of differences with the control group, $p<0.05$. [&]Reliability of differences with the Group I, $p=0.007$. CI: Confidence interval.

Discussion

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. In our opinion, both these groups can be considered as patients with a preclinical stage of kidney damage and a high risk of nephropathy development. This situation is similar to the other, observed in patients with diabetes mellitus, in whom, according to the literature, microalbuminuria is often accompanied by increased eGFR and is considered as the initial stage of diabetic nephropathy. It was also shown that the initial stage of kidney damage in the case of hypertension is also manifested by an increase in eGFR, while at later stages – by decline [23]. Thereby, eGFR reflects early, intermediate, and later stages of kidney damage, but changes in eGFR are not equivalent. The decrease in eGFR in combination with the diagnostic criteria of toxic nephropathy, proposed by Luzhnikov and Kostomarov, reveals only the late stage of kidney damage when the function of a significant part of nephrons is lost. 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. 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.

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