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MYO-INOSITOL OXYGENASE AS A NOVEL MARKER IN THE DIAGNOSIS OF ACUTE KIDNEY INJURY

MIO-INOZITOL OKSIGENAZA KAO NOVI MARKER ZA DIJANGOZU AKUTNOG BUBREŽNOG OŠTEĆENJA

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Summary

Background: Due to the lack of diagnostic efficiency of serum creatinine in acute kidney injury (AKI), there is a pressing need to develop novel diagnostic markers. Therefore, in this study, we evaluated myo-inositol oxygenase (MIOX), neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C in terms of their applicability in the diagnosis of AKI.
Methods: We enrolled a total of 39 AKI patients and 38 healthy controls in the study. We compared the levels of serum MIOX, NGAL and cystatin C between the two groups.
Results: We found that the concentrations of serum creatinine, blood-urea nitrogen, MIOX and cystatin C were higher in the AKI group. According to the receiver operating characteristic analysis, the area under the curve (AUC) values were 0.694 (95% CI 0.579–0.794) for MIOX and 0.976 (95% CI; 0.912–0.997) for cystatin C. For MIOX, when the cut-off concentration was set to 77.3 pg/mL, the diagnostic sensitivity and specificity were found to be 53.8% (95% CI; 37.2–69.9) and 81.5 (95% CI; 65.7–92.3), respectively. For cystatin C, at the cut-off value of 14 mg/L, the diagnostic sensitivity and specificity were 94.8% (95% CI; 82.7–99.4) and 94.7 % (95% CI 82.3–99.4), respectively.
Conclusions: The measurement of serum MIOX and cystatin C levels is valuable for the diagnosis of AKI. Further research is needed for the evaluation of the potential use of MIOX as a kidney-specific enzyme in the early diagnosis of AKI.

Keywords: acute kidney injury, myo-inositol oxygenase, neutrophil gelatinase-associated lipocalin and cystatin C

Kratak sadržaj

Uvod: Zbog manjavosti serumskog kreatinina za dijagnostičku efiksanost akutnog bubrežnog oboljenja (ABO), nameće se potreba za otkrivanjem novih dijagnostičkih markera. Iz tog razloga ovde je procenjivana primena mio-inozitol oksigenaze (MIOX), neutorfilne želatinaze-udružene sa lipokainom (NGAL) i cistatina C za dijagnostikovanje ABO.
Metode: Izučavano je ukupno 39 AKI pacijenata i 38 kontrolnih ispitanika. Upoređivani su nivoi MIOX, NGAL i cistatina C između dve grupe.
Rezultati: Nađeno je da su koncentracije serumskog kreatinina, ureje, MIOX i cistatina C bile više u AKI grupi. Prema ROC analizi, površine ispod vrednosti krive (AUC) iznosile su 0,694 (95% CI; 0,579–0,794) za MIOX i 0,976 (05% CI; 0,912–0,997) za cistatin C. Za MIOX, kad je cut-off vrednost podešena na 77,3 pg/mL, nađeno je da su dijagnostička osetljivost i specifičnost bile 53,8% (95% CI; 37,2–69,9) i 81,5 (95% CI; 65,7–92,3). Za cistatin C pri cut-off vrednosti od 14 mg/L, dijagnostička osetljivost i specifičnost su bile 94,8% (95% CI; 82,7–99,4), odnosno 94,7/ (95%CI 82,3–99,4).
Zaključak: Merenje serumske MIOX i nivoa cistatina C je značajno za dijagnostikovanje AKI. U daljim istraživanjima treba procenjivati potenicijalnu primenu MIOX kao bubrežnog specifičnog enzima za ranu dijagnozu AKI.

Ključne reči: akutno bubrežno oboljenje, mio-inozitol oksigenaza, neutrofilna želatinata-udružena sa lipokainom, cistatin C

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Introduction

Acute kidney injury (AKI) is a serious clinical problem due to its high morbidity and mortality (1). Therefore, early diagnosis and treatment for this condition is of critical importance. Currently, a diagnosis of AKI is based on the level of the plasma creatinine. However, creatinine is an insensitive and non-specific marker that is affected by several variables such as age, gender, muscle density and hepatic functions. Furthermore, an increase in the level of creatinine is only observable days after a kidney injury and when 50% or more of the kidney functions are affected (2). Therefore, the search for alternative markers for early diagnosis of AKI has been increasing. Some markers that have been suggested to date are neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), liver-type fatty acid–binding protein (L-FABP), interleukin 18 (IL-18), insulin-like growth factor binding protein 7 (IGFBP7), tissue inhibitor of metalloproteinase 2 (TIMP-2), and calprotectin (S100A8/9) (3).

NGAL, a 25-kDA protein part of the lipocalin superfamily, was first discovered in activated neutrophils and renal tubular cells. The existence of NGAL can be ascertained from urine and plasma samples. This method of measuring serum or urine NGAL following possible renal injury has the main advantage of that there are relatively rapid changes over time in the serum NGAL levels, which usually occur within several hours after the injury (4). Myo-inositol oxygenase (MIOX, EC 1.13.99.1) is the first rate-limiting enzyme of myo-inositol catabolism (5,6) and is mostly observed in the kidney (7). The oxidative cleavage of myo-inositol (MI) is catalyzed by myo-inositol oxygenase (MIOX), a significant process in MI catabolism that produces D-glucuronic acid (8). This enzyme was first detected in the extracts from rat kidney and purified from pig kidney in the 1980s (5). However, our literature review showed that there have only been a limited number of studies investigating this enzyme in the last few decades. A recent significant study of Gaut et al. (9) verified the specificity of MIOX to the proximal renal tubules using the Western blot technique, thus for the first time demonstrating that this enzyme could be used as a marker for the early diagnosis of AKI (9).

Protein cystatin C inhibits cysteine protease and is synthesized by all nucleated cells at a constant rate. This process is not affected by inflammation, catabolism or diet. In terms of molecular weight and isoelectric point, cystatin C is freely filtrated in the glomerulus, resorbed, and completely degraded by the proximal tubule cells; thus, indirectly demonstrating the rate of glomerular filtration in its plasma concentration (10).

Some studies published in the literature suggest that cystatin C is superior to creatinine in terms of the diagnosis of AKI (11) whereas other researchers disagreed with the superiority of cystatin C and even argued that creatinine is actually a more effective diagnostic tool (12).

In this study, we aimed to further investigate the potential use of MIOX enzyme as a diagnostic marker specific to AKI, which has only been reported in one study (9). Furthermore, in order to shed more light on the debate regarding the superiority of cystatin C, we compared the effectiveness of MIOX, creatinine, NGAL and cystatin C in diagnosing AKI. Considering the significance and unavailability of markers in AKI diagnosis, we found it surprising that rare renal specific enzymes such as MIOX have only received limited attention from researchers.

Material and Methods

The study group consisted of 39 patients (19 female and 20 male) that were diagnosed with AKI by the same nephrologist in the polyclinic and emergency service of our hospital between December 2015 and June 2016. AKI etiology of the patients was different, there were hypovolemia/prerenal factors in 24 patients, renal pathology in 9 patients and postrenal/obstructive factors in 6 patients. In addition, a control group of 38 individuals (18 female, 20 male) was formed with healthy volunteers. The exclusion criteria were the presence of acute diseases such as myocardial infarction, sepsis, and acute respiratory distress syndrome and chronic diseases including diabetes mellitus, rheumatic and cardiovascular diseases. The study was approved by the clinical research ethics committee of Erzincan University, Turkey (number: 44495147-050.01.04-E.44576, date: 16/11/2015).

An AKI diagnosis can be determined by a ≥3.0 mg/L increase in serum creatinine within 48 hours, a ≥1.5-fold increase compared to a known or assumed baseline or a decrease in urinary output to <0.5 mL/kg/h over 6 hours (13).

The blood samples were collected and immediately centrifuged. The extracted serum samples were kept at –80 °C until use in biochemical tests. The MIOX and NGAL levels were measured using the ELISA method [Cusabio Human inositol oxygenase (MIOX) ELISA Kit and Sun Red Human (NGAL) ELISA kit]. The results were recorded in pg/mL and ng/mL.

A Beckman Coulter Olympus AU2700 system was used to analyze the total concentrations of serum creatinine, blood urea nitrogen (BUN) and cystatin C. The results were recorded in mmol/L for creatinine and BUN, mg/L for cystatin C.

Statistical analysis

Data were analyzed using Excel and MedCalc. Descriptive statistics (mean, standard deviation, medi-

an, minimum, maximum, number) were generated for the two groups. The normality assumption was checked using the Kolmogorov-Smirnov test. Differences between the two groups were evaluated by the independent t-test when the assumptions of this parametric test were met. The p-value of <0.05 was considered as statistically significant.

Results

There was no statistical difference between the AKI and control groups in terms of gender, weight and height. However, the age range of the study group was found to be significantly higher than the control group (*Table I*).

The serum creatinine, BUN, MIOX and cystatin C values were significantly higher in the AKI group compared to the control group (*Table II*). The receiver operating characteristic (ROC) curves were generated for creatinine, BUN, MIOX and cystatin C values for the diagnosis of AKI. The area under the curve (AUC) was calculated as 0.694 for MIOX [95% confidence interval (CI); range: 0.579–0.794], 0.976 for cystatin C (95% CI; range: 0.912–0.997), 0.984 for BUN (95% CI; range:0.924–0.999) and 0.993 for creatinine (95% CI; range: 0.939–1.0) (*Figure 1*). When the cut-off value was taken as 77.3, the diagnostic sensitivity and specificity of MIOX for AKI were 53.8 (95% CI; range: 37.2–69.9) and 81.5 (95% CI; range: 65.7–92.3), respectively. For cystatin C, at the cut-off value of 14, the sensitivity and specificity for AKI were found to be 94.8 (95% CI; range: 82.7–99.4) and 94.7 (95% CI; range: 82.3–99.4). For BUN, at the cut-off value of 13.21, the sensitivity and specificity for AKI were found to be 96.2 (95% CI; range: 87–99.5) and 83.3 (95% CI; range: 67.2–93.6). For creatinine, at the cut-off value of 0.109, the sensitivity and specificity for AKI were found to be 98.1 (95% CI; range: 89.9–1.0) and 91.7 (95% CI; range: 77.5–98.2). The serum NGAL levels were similar in both groups.

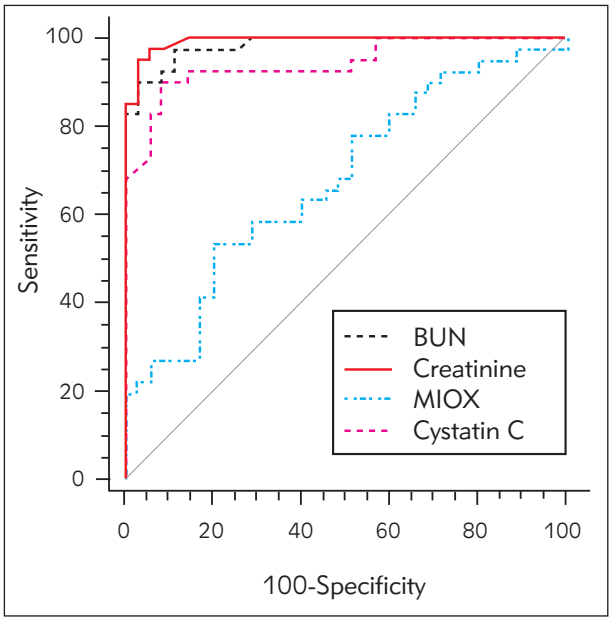

Figure 1 ROC curve analysis of serum creatinine, BUN, cystatin C and MIOX for the diagnosis of AKI (AUC; for creatinine; 0.993, BUN; 0.984, cystatin C; 0.976, MIOX; 0.694).

Table I Comparison of demographic data of AKI and control groups.

Demographic data	Control (mean±SD)	AKI (mean±SD)	p- value
Age	41±15	66±19	<0.0001[a]
Female (%)	18 (47.3)	19 (48.7)	0.9127
Male (%)	20 (52.7)	20 (51.3)	0.5895
Weight (kg)	72.4±11.3	71±10.6	
Length (cm)	165.3 ±5	167.9±9	0.1724

a; p<0.0001

Table II Comparison of biochemical data between AKI and control groups.

Parameter	Control (mean±SD)	AKI (mean±SD)	p-value
BUN, mmol/L	8.85±2.7	46.17±30.3	<0.0001[a]
Creatinine, mmol/L	0.079±0.00	0.265±0.13	<0.0001[a]
NGAL, ng/mL	177±81	189±81	0.5326
Cystatin C, mg/L	10±3	26±12	<0.0001[a]
MIOX, pg/mL	60±32	101±69	0.0016[b]

a; p < 0.0001, b; p <0.05.

Discussion

This is the second study in the literature that reports on the significance of MIOX enzyme for the diagnosis of AKI [for the first study, see (9)]. The renal specificity of the MIOX has been known for a long time (7); however, to date, it has not attracted the deserved attention from researchers. Considering the significance and lack of diagnostic markers for the early diagnosis of AKI, it is clear that MIOX is a very important enzyme that needs to be investigated. Therefore, similar to the first and only other study in the literature (9) demonstrating the role of MIOX in AKI diagnosis, we have confirmed the significance of MIOX.

Gaut et al. (9) conducted their study in two main phases. In the first phase, renal ischemic damage was induced in mice over 30 minutes. The serum MIOX values that were measured 24 hours later were found to be significantly higher than the healthy control group. In the second phase, patients that were diagnosed with AKI based on increased serum creatinine and urine output according to the AKI network criteria (14) were evaluated in terms of their MIOX values. The MIOX levels of the patients that were measured three days previously were found to precede the increase in creatinine levels by two days. In the same study, Gaut et al. (9) used the Western blot technique to demonstrate that MIOX is a renal-specific proximal tubule protein. Similarly, in the current study, we obtained significantly higher MIOX values in the AKI group. Also, the diagnostic sensitivity and specificity of MIOX for AKI were 53.8 % and 81.5 % respectively. Of course this result is important to use MIOX in the diagnosis of AKI. However, due to MIOX is an enzyme of proximal kidney tubules, all kinds of tubular lesions are likely to cause MIOX elevations. It is clear that these conditions such as aminoglycoside administration, tubular toxicity, pyelonephritis should be tested in order to see a real life ROC curve.

Although several molecules including NGAL, KIM-1, IL-18, IGFBP7, liver type fatty acid-binding protein 1 (FABP1) and N-acetyl-β-D-glucosaminidase have been reported to have a role in the diagnosis of AKI, none has been put into routine clinical practice particularly due to the inadequacies and problems regarding their method of measurement (3, 9, 11, 15). Compared to the alternatives, MIOX seems to be a more promising potential marker for a number of diseases considering the results reported in other studies. For example, Prabhuet al. (8) investigated the role of MIOX in diabetic nephropathy and reported the presence of a positive feedback mechanism that upregulated MIOX, in which a product of MI catabolism, xylitol, induced the expression of MIOX through the pathway of glucuronate-xylulose (8). In another study, the overexpression of MIOX in diabetic nephropathy was found to cause glucose-induced intermediaries and activate kinases that modulated different transcription factors. The authors suggested that MIOX activity was regulated by post-translational modifications (16).Yang et al. (6) suggested an association between polymorphism (rs761745) in the promoter region of the MIOX gene and type 1 diabetes mellitus (6). A recent study from Sun et al. (17) argued that MIOX under high glucose ambience exacerbates renal injury during the progression of diabetic nephropathy following the generation of excessive reactive oxygen species (ROS). An other newly research (18) claimed that MIOX overexpression aggravates, whereas MIOX gene deficiency protects against, cisplatin-induced AKI. So in our study, the cause of high level of MIOX in AKI patients may be related to increased to ROS. Because it is also known that there is an association with oxidative stress and AKI (19, 20).

The authors diagnosed AKI based on cystatin C, BUN and creatinine levels, and found the formers (AUC=0.976 and 0.984) to be as effective as the latter (AUC=1.0) with no significant difference between the three (p=0.1319). However, there are controversial reports regarding the significance of these levels for early diagnosis. For example, Haase-Fielitzet al. (11) evaluated the NGAL and cystatin C level in patients on their admission to intensive care following heart surgery, and found them to be superior to creatinine for the diagnosis of AKI. However, the authors reported that the NGAL level increased before the increase in the serum creatinine, and as the serum creatinine level increased 24 hours after surgery, the NGAL levels started to decrease (11). Similarly, in the present study, the AKI diagnosis was made according to the creatinine levels of the patients and by the time the creatinine levels increased, the serum NGAL levels were reduced, thus resulting in non-significant differences between the AKI and control groups.

In their study on contrast-induced AKI, Padhy et al. (20) observed increased serum NGAL values in the AKI group following angiography with the maximum value being obtained at the 4^{th} hour, which was also significantly different from the measurement at hour 0. The NGAL values started to decrease after 24 hours but remained significantly higher than the baseline. At the 48^{th} hour, the NGAL value was reduced to the baseline. In contrast, in the AKI group serum cystatin C reached a maximum level at 24 hours and started to decrease at 48 hours maintaining its significant difference compared to the baseline. However, in the control group, the serum levels of NGAL and cystatin C were not found to significantly differ. These findings are consistent with the results of our study in terms of demonstrating that at the time when the creatinine level significantly increased and the AKI diagnosis was made, the NGAL level had already started to decline from its maximum value and returned to the baseline whereas cystatin C continued to be on an increase (21).

A recent study has shown that in patients that had undergone heart transplantation, the serum cystatin C levels at hour 3 were significantly higher in those that developed AKI. In addition, the authors concluded that a persistent increase in cystatin C could predict one-year mortality (10). In another study, patients with a high risk of AKI were evaluated and cystatin C was found to be superior to creatinine since it allowed the diagnosis of AKI to be made 1-2 days before creatinine (22). In contrast, Spahillari et al. (12) conducted a study in multiple centers with a total of 1,150 patients that had undergone heart surgery. The authors measured the cystatin C levels before and five days after the surgery and found that these levels were no more effective than creatinine in the early diagnosis of AKI.

In the present study, we measured the MIOX level at the time of diagnosis of AKI based on the creatinine levels. However, as mentioned above, creatinine is a non-specific marker that begins to increase days after kidney injury. Therefore, in future studies, patients with the potential to develop AKI should be closely monitored by measuring their serum and urine MIOX levels before the increase in creatinine. This would demonstrate the role of MIOX levels in early diagnosis and such an evaluation would probably produce significant results in the early period due to the half-life of MIOX. In AKI, it is not always possible to know when damage has been incurred; therefore, there is a possibility that by the time creatinine levels have increased, MIOX may have already increased and declined. The renal specificity of MIOX indicates its potentiality for use in the differential diagnosis of AKI. There is also a need for further investigation into the MIOX levels in systemic cases in which, sepsis, multiple organ failure and shock affect the kidneys.

Conclusion

MIOX and cystatin C were found to be significant parameters in the diagnosis of AKI. MIOX presents as a promising potential biomarker that needs to be further investigated in terms of its role in the early diagnosis of AKI.

Limitations

Serum creatinine level was used for AKI definition. One of the other limitation of this study is the fact that healthy individuals were used as the control group, while patients from acute care settings such as emergency service and ICUs were included in the AKI group. It is possible that the increase in MIOX levels could be effected to nonspecific acute illness. In future studies, it may be preferable to include acutely ill, but non-AKI patients as a control group. Also we want to note that the Cusabio ELISA assay was not intended for clinical use because of has not been validated at all.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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