IJKD KIDNEY DISEASES

The Application of Urinary NGAL Measurement for Early Detection of AKI in Hospitalized Patients with Poisoning

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Original Paper

Introduction. Early diagnosis of acute kidney injury is critical for decision-making. Neutrophil gelatinase-associated lipocalin (NGAL) is a biomarker introduced for early detection of acute kidney injury (AKI). We evaluated urinary NGAL level in hospitalized patients due to poisoning as a predictor of AKI.

Methods. We studied patients with poisoning due to various causes. Urinary NGAL and urine creatinine levels were measured. Serum creatinine levels were measured for all patients at baseline and after 24 and 48 hours. Then, a ROC curve developed for urinary NGAL, and cutoff point and accuracy of urinary NGAL test were determined.

Results. Ninety hospitalized patients with acute poisoning were consecutively recruited into the study over an eight-months period. With the gold standard test (i.e., serum creatinine measurement), 21 patients were diagnosed with acute kidney injury (AKI) and 69 with non-AKI, whereas according to ROC curve, at a cutoff point of 110 ng/ml, urinary NGAL with an 81% sensitivity and 91.3% specificity distinguished 23 patients with AKI and 67 with non-AKI. The false positive and false negative values of urinary NGAL test were 8.7% and 19%, respectively. The positive predictive value and negative predictive value of urinary NGAL were estimated to be 73.9% and 94%, respectively.

Conclusion. Urinary NGAL test, with an AUC of ROC curve of approximately 90% and a sensitivity of 81%, can be used for early detection of AKI. It has a high specificity (91.3%), indicating that the percentage of false positive cases (8.7%) will be small.

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INTRODUCTION

Acute kidney injury (AKI) is characterized by a rapid decrease in the glomerular filtration rate and increase in retention of waste metabolic materials. According to AKIN classification, AKI is diagnosed when one or more of the following conditions exist: 1) an increase in serum creatinine of at least 0.3 mg/dL within 48 hours, 2) at least 50% increase over baseline amount within a week, or 3) a decrease in urinary output to 0.5 mL/kg/h for more than 6 hours.[1]

The diagnostic methods used for AKI include measuring serum creatinine and urea, urinary findings, radiological methods, and kidney biopsy. Many new biomarkers have been discovered for detection of kidney damage. One of these biomarkers is neutrophil gelatinase associated lipoprotein (NGAL).

Human NGAL was first derived from neutrophils, but it can also be found in small amounts in other

tissues, such as epithelial cells of the proximal tubules of the kidney, and the digestive and the respiratory systems. NGAL is also seen in adenomas and inflamed colon cells, breast adenocarcinoma, and epithelial cell carcinoma of the urinary tract.[2,3] NGAL is secreted from secondary granules of activated neutrophils and therefore, its plasma levels are increased during inflammation or infection. It belongs to the lipocalin family which are small secretory proteins identified by their ability to bind to small and hydrophobic molecules.[4] Many of the lipocalins bind to specific surface receptors of some cells, but no specific NGAL receptor has been identified. Human NGAL consists of a polypeptide chain with a di-sulfide bond on amino acid 178 having a molecular weight of 22 kilodalton.[5]

Considering that one of the sources of NGAL production is the proximal tubular epithelial cells, urinary NGAL has recently been considered as a marker of kidney injury in numerous diseases.[6] Urinary NGAL can reveal kidney injury before any changes in serum urea or creatinine. The use of urinary NGAL has a very beneficial role in early detection of AKI and, consequently; preparing for the prevention and the management of its complications. In addition, the urinary NGAL test is more cost effective than serum creatinine test; since it is less invasive and lower in cost. As a result, urinary NGAL has been remarkably utilized in ICU patients for early detection and treatment of AKI and attaining better outcome.[7] Likewise, It has been shown that NGAL can be used for early diagnosis of AKI in patients undergoing cardiac surgery or other high risk groups.[8,9] It is noteworthy that urinary NGAL can be detected at a time that kidney injury is reversible and its level indicates the severity of the kidney injury.[10]

Since the amount of Urinary NGAL is elevated in AKI patients prior to any increase in urea and creatinine, it can be used for early diagnosis and rapid onset of treatment of AKI. The urinary NGAL was measured in the hospitalized patients with poisoning to assess its capability in AKI diagnosis.

MATERIALS AND METHODS

From March 2018 to November 2018, all patients who had been hospitalized due to various causes of poisoning in the Emergency Department of Khorramabad Shahid Rahimi Hospital for more than six hours consecutively participated in the study. The study was a descriptive cross-sectional which included the patients who were otherwise healthy before admission for poisoning and excluded the known cases of acute or chronic kidney injury. Urine and blood samples were drawn within 6 to 12 hours after exposure to the poisoning agents. Other blood samples for creatinine were prepared after 24 and 48 hours of exposure to the agent. Then, according to the results of the gold standard test and using AKIN classification criteria, patients were divided into two groups, AKI and non-AKI.

To evaluate the accuracy of urinary NGAL in the prediction of AKI, as well as to determine the sensitivity and the specificity of the NGAL test, the ROC curve has been established.

Urinary NGAL was measured by NGAL ELISA Kit (human), Cat. No. KIT 036 manufactured by BIOPORTO DIAGNOSTICS A/S, Denmark. The results were analyzed using SPSS version 24. Descriptive statistics are presented as frequencies and percentages for categorical, and means and standard deviations for continuous variables. The Number of participants is large enough to assume normality and apply parametric tests. The significance level for statistical tests is .05.

The study proposal was approved by the Committee of Ethics, Deputy of Research and Technology of Lorestan University of Medical Sciences on 2017-11-20 with code number IR.LUMS.REC.1396.325. Deputy of Research and Technology of Lorestan University of Medical Sciences funded the study.

RESULTS

Ninety patients with poisoning, including 55 males (61.1%) and 35 females (38.9%), were studied. The mean age of patients was 27.11 ± 10.97 years. Patients with CKD, or with diseases predisposing to CKD such as diabetes were excluded from the study. At the time of admission, one patient had high blood pressure (140/95), and 18 patients (7 females and 11 males) had blood pressures within hypotensive range. Table 1 shows basic characteristics of the patients participated in this study. In Table 2, means and standard deviations for the laboratory biochemical tests for all studied patients are demonstrated. The values of laboratory tests were compared in both sexes and no statistically significant differences were demonstrated. Urinary NGAL was not correlated

Table 1. Basic Characteristics of the Patients

Variable	Female	Male	Total
Sex	35 (38.9%)	55 (61.1%)	90 (100%)
Age (Mean±SD)	25.31 ± 11.32	28.25 ± 10.69	27.11 ± 10.97
Poisoning type			
Oral	35 (38.9%)	55 (61.1%)	90 (100%)
Other	0	0	0
History of Antineurotic and/or Antipsychotic	9 (43%)	12 (57%)	21 (100%)
History of CKD	0	0	0
Severity of Poisoning	Moderate to Severe	Moderate to Severe	Moderate to Severe
Level of Consciousness at the Time of Admission (no. of Patients)			
Fully Awake	19 (54%)*	27 (49%)†	46 (51%)
Conscious but Drowsy	9 (26%)	18 (33%)	27 (30%)
Unconscious	7 (20%)	10 (18%)	17 (19%)
Need for ICU	9 (47%)	10 (53%)	19 (100)

*Percentage of All Females
†Percentage of all Males

Table 2. Means and Standard Deviations of Laboratory Tests for All Studied Patients

Laboratory Test	Mean ± SD*	Lowest Value	Highest Value
Serum Creatinine3†	1.2 ± 0.38	0.6	2.8
Urinary Creatinine	85.13 ± 64.2	3.28	285
Urine NGAL	116.15 ± 182.86	1	1056

*Serum creatinine3 measured after 48 hours of exposure.
†Calculated for All Ninety Patients

with age, both for the whole patients and for AKI and non-AKI; separately. In order to identify if there were any differences for laboratory tests values between AKI and non-AKI patients, the values were compared using independent t-test (Table 3).

One of the main objectives of this study was to determine the accuracy and predictive value of urinary NGAL in detecting acute kidney injury in patients while compared with gold standard (i.e., serum creatinine) test results. Hence, the Receiver Operating Characteristic (ROC) curves were constituted to determine the appropriate cutoff point for the urinary NGAL test (Figure).

The Area under the ROC curve (AUC) was 0.90 with (95% CI: 0.82 to 0.95) and a significance level $P < .001$, which indicates the accuracy of the curve is "good".

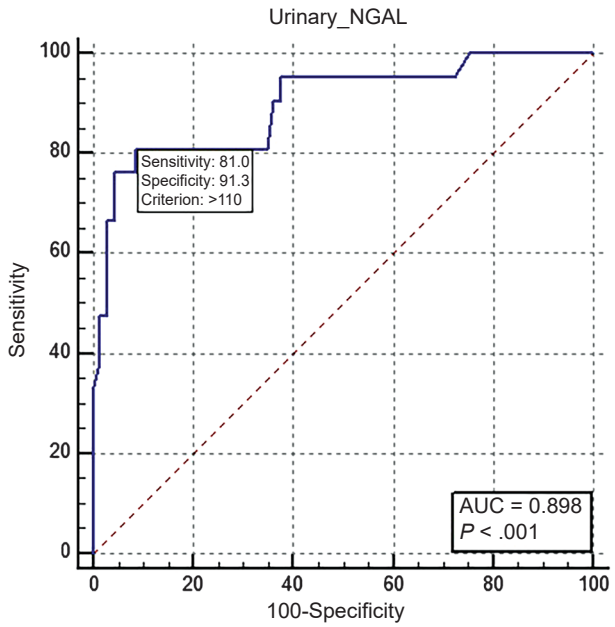
According to Youden Index, which is a summary measure of the ROC (Receiver Operating Characteristic) curve, the optimal threshold value (i.e., the most suitable cutoff point) at a sensitivity of 80.95 and a specificity of 91.30 is 110 ng/mL for urinary NGAL in our study.

Table 4 compares the diagnostic accuracy of NGAL with serum creatinine, the gold standard test for diagnosis of AKI. Sensitivity, specificity, positive predictive value (PPV) and negative predictive

Table 3. Comparison of Laboratory Test Results in AKI and Non-AKI Patients

Variable	Mean ± SD		T Statistic	*P* (2-tailed)*	Mean Difference	Std. Error of Difference
	AKI	Non-AKI				
Urine NGAL	325.86 ± 276.18	52.33 ± 60.15	1.75	.00	273.53	60.70
Urinary Cr	97.17 ± 68.13	81.46 ± 63.04	1.90	.33	15.71	16.01
Serum Cr1†	1.04 ± 0.21	1.02 ± 0.26	.51	.74	.02	.06
Serum Cr2‡	1.42 ± 0.34	1.05 ± 0.22	.80	.00	.37	.06
Serum Cr3§	1.70 ± 0.39	1.06 ± 0.23	.57	.00	.64	.07

*Independent t-test, df = 88
†Serum creatinine measured within 6 to 12 hours of exposure.
‡Serum creatinine measured 24 hours after exposure.
§Serum creatinine measured 48 hours after exposure.



It shows the Receiver Operating Characteristic (ROC) curve analysis.

Table 4. Urinary NGAL Test Results for Diagnosis of AKI in Contrast to Serum Creatinine (i.e., Gold Standard Test for AKI)

Urinary NGAL Result	Serum Creatinine3* Result		
	AKI	Non-AKI	Total
AKI	17	6	23
Non-AKI	4	63	67
Total	21	69	90
Sensitivity = (17 ÷ 21) × 100 = 81%		95% CI: 58.1 to 94.55	
Specificity = (63 ÷ 69) × 100 = 91.3%		95% CI: 82.03 to 96.74	
Positive Predictive Value = (17 ÷ 23) × 100 = 73.9%		95% CI: 56.20 to 86.22	
Negative Predictive Value = (63 ÷ 67) × 100 = 94.03%		95% CI: 86.7 to 97.45	
Percentage of False Positives (1 – Specificity) = 1% - 91.3% = 8.7%			
Percentage of False Negatives (1- Sensitivity) = 1% - 81% = 19%			

*Serum creatinine3, serum creatinine measured after 48 hours of exposure.

value (NPV) are presented in the table. The PPV of urinary NGAL testing is 73.9%, indicating that the probability of being affected by AKI in patients who are positive for their urinary NGAL is 73.9. Also, the NPV of urinary NGAL testing is 94, which is a considerable value demonstrating that patients with negative urinary NGAL result are not affected by AKI.

Overall, NGAL measurement, despite 19% false negatives, with a good AUC can be used for early detection of AKI in poisoning. The high specificity demonstrates that the test can be very helpful in distinguishing non-AKI patients.

DISCUSSION

We assessed the diagnostic accuracy of urinary NGAL test in early detection of AKI in hospitalized patients due to poisoning. Our study results show that urinary NGAL is elevated in AKI patients within several hours of admission, much earlier than serum creatinine. With an AUC of the ROC curve of 0.90 and a good sensitivity and specificity (81% and 91.3%, respectively), it could properly predict AKI. During the recent decade, urinary NGAL has been studied for various purposes including acute and chronic situations.

There are numerous studies that indicate the accuracy of both serum and urinary NGAL in early diagnosis of AKI in various clinical settings. For example, Wang *et al.* divided 153 patients with AKI after cardiac surgery as being alive or deceased. Serum NGAL was higher in deceased group and the more elevated NGAL level correlated with poorer prognosis.[11] In severe sepsis or septic shock, after general surgery, and immediately after lithotripsy, NGAL increased significantly if AKI has occurred.[12-14] Even NGAL could have predicted death or the need for dialysis in AKI patients.[15] Likewise, it was revealed in our study that NGAL could detect AKI in patients with critical disorders without delay. Of course, this is not always the case for all patients (because of false negatives) but regarding the importance of early diagnosis of AKI for preventing irreversible complications, it will be very beneficial in many situations. NGAL has been studied in chronic kidney injury (CKI), too. Urinary NGAL can be used as a marker for determining the extent of kidney injury in multiple myeloma.[16] Likewise, in progression of CKI to ESRD, the urinary NGAL increased gradually.[17] However, in some settings, NGAL has not acted adequately. For example, although NGAL is a acceptable predictor for detecting preeclampsia and its level significantly correlates with the amount of proteinuria, it cannot appropriately predict AKI in preeclampsia.[18,19] In a study by Singer E *et al.*, urinary NGAL levels were evaluated in 161 patients with AKI. Sixteen patients were excluded from the study due to post-renal obstruction. Of the remaining 145, 75 were intrinsic AKI and 32 were pre-renal AKI and 38 were unclassified. Urinary NGAL levels increased in both the pre-renal and the intrinsic AKI, and its level in the intrinsic AKI was significantly higher than in the prerenal.[20] In

our study, nearly all of the patients were affected by intrinsic AKI; nephrons as the dominant source of NGAL might have been considerably damaged to produce it.

Although there are studies in which sensitivity (65%), specificity (65%), and AUC (0.70) of the urinary NGAL are not considerably increased, NGAL can be reliably measured in clinical urine samples and its value is elevated with AKI.[21] In our study, AUC, sensitivity, and specificity have higher values than this study, indicating higher diagnostic accuracy for NGAL.

In a multicenter study of 1635 subjects, Nickolas and associates revealed that urinary NGAL provided an AUC of 0.81 (95% CI: 0.76 to 0.86) for the prediction of AKI and its values were significantly different and increased gradually in the following order: patients with no AKI, patients with CKD, patients with pre-renal AKI, patients with intrinsic AKI.[22] Again, our study has a higher AUC and therefore, is more accurate for the prediction of AKI.

El Gendy and colleagues studied 80 patients in pediatric ICU. They showed that serum NGAL at a cut-off point of 155 ng/mL, sensitivity of 100%, specificity of 89.8%, PPV of 68.8%, and NPV of 100% acts as a sensitive marker for AKI diagnosis in these patients. In their study, NGAL has a NPV more diagnostic than PPV for AKI.[23] Similarly; in our study NPV is more diagnostic than PPV. Although our study sensitivity is less than El Gendy's, it is at a lower cut-off point.

Meta-analysis of 37 systematically reviewed studies in terms of AUC, sensitivity and specificity illustrated that urine NGAL, if measured during the first 6 hours after admission or surgery, could optimally detect AKI in children at a cut-off of 50 ng/mL.[24]

As is observed in the above studies, there are different cutoff points for urinary NGAL to detect AKI. It may be due to different study subjects, laboratory kits, sample sizes, and different time intervals of urine sampling from the exposure to the predisposing factor, or other factors such as different AUC at the expense of different sensitivity and specificity. A limitation of our study was that due to different referral time of patients to the hospital, the planning for urinary NGAL sampling at an exact equal interval from exposure for all patients was not feasible.

CONCLUSION

The urinary NGAL test can be used for early detection of AKI in poisoning. The high specificity and the low false positive rate demonstrate that the test can be very helpful in distinguishing non-AKI patients. The clinical applications (strengths and weaknesses) of urinary NGAL test for early detection of AKI needs to be investigated in broader studies. (i.e., with larger sample sizes and multiple consecutive measurements)

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