

Assessment of the Diagnostic Validities of Serum NGAL, KIM-1, and L-FABP in Patients With Chronic Kidney Disease

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Abstract

Introduction: Chronic kidney disease (CKD) is one of the most threatening and important disorders worldwide in both industrial and developing nations. In addition, neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid-binding protein (L-FABP), and kidney injury molecule-1 (KIM-1) are three factors suggested as diagnostic and prognostic biomarkers in CKDs. Considering the lack of enough efficiency of the creatinine in the prognosis of the CKD, the present study aimed to assess the relationship between these three factors and CKD occurrence and determine if they could be considered valid biomarkers in this regard.

Materials and Methods: The present case-control study was designed enrolling 42 patients with confirmed CKD referring to the Imam Khomeini hospital of Kangan. The participants were 42 years old and gender-matched healthy counterparts. Blood samples were obtained, and then NGAL, KIM-1, and L-FABP were determined by the enzyme-linked immunosorbent assay using commercial kits (Bioassay Technology Laboratory). Finally, the serum creatinine was detected by applying Jaffe's method.

Results: Based on the results, significant differences were found in the serum levels of all four factors between CKD patients and the control group. More precisely, the serum levels of NGAL ($P < 0.0001$, specificity: 87.6%, sensitivity: 79.3%, and the area under the curve, AUC: 0.89), L-FABP ($P < 0.0001$, specificity: 83.3%, sensitivity: 78.3%, and AUC: 0.86), KIM-1 ($P < 0.0001$, specificity: 85.7%, sensitivity: 78.6%, and AUC: 0.88), and creatinine ($P < 0.0001$) were significantly higher in individuals with CKDs in comparison with controls. Eventually, the serum levels of NGAL, L-FABP, and KIM-1 were significantly correlated with each other in both patient and control groups ($P < 0.0001$).

Conclusion: In general, NGAL, L-FABP, KIM-1, and creatinine could be used as independent biomarkers for the diagnosis of CKD. Moreover, the measurement of NGAL, L-FABP, and KIM-1 altogether could be a valid assessment for the diagnosis of CKD.

Keywords: Chronic kidney disease, Liver-type fatty acid-binding protein, Kidney injury molecule-1, Neutrophil gelatinase-associated lipocalin

Introduction

Chronic kidney disease (CKD) affects a vast number of individuals worldwide as is known as the 12th most prevalent cause of morbidity in patients.^{1,2} It seems that patients with CKD are more likely to succumb to cardiac failure than the end-stage renal disease. Diabetes and hypertension can be observed in the final stages of CKD as well.³ Because of having a high impact on public health and involving many patients, precise and reliable biomarkers are needed for the early diagnosis of CKD. It is noteworthy that serum creatinine serves as a biomarker

regarding evaluating renal impairment and the progression of CKD. Although creatinine is a proper index for assessing glomerular filtration, it cannot be used as a biomarker for kidney failure^{4,5} since its sensitivity is affected by gender, muscle mass, race, and medications.⁶⁻⁸

Neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid-binding protein (L-FABP), and kidney injury molecule-1 (KIM-1) have been suggested as proper biomarkers for the early detection of CKD.⁹⁻¹¹ NGAL, as a 25 KD protein, is mainly produced in renal

tubular cells. Further, it can be detected at low levels in the urine and plasma. Experimental studies reported NGAL as one of the key genes with high levels of mRNA expression in the kidney.⁹ Furthermore, previous research introduced NGAL as a bacteriostatic component involved in the innate immune pathway. Moreover, this molecule has attracted remarkable attention since new results suggest NGAL as a prognosis biomarker in kidney disease.⁴

Moreover, L-FABP is expressed in the proximal epithelial cells of the renal and can bind to free fatty acids. In the kidney, the mRNA level of LFABP is significantly up-regulated in the proximal area of renal tubules. Additionally, urinary LFABP (u-LFABP) is mainly regarded as a urinary tubular biomarker associated with kidney damage.⁵ It has been reported that the increased levels of u-LFABP are dramatically correlated with renal tubulointerstitial damage because of the excessive reabsorption of FFA into the proximal area of renal tubules that affects tubulointerstitial damage.¹²

Similarly, KIM-1 is massively unregulated in proximal kidney cells after kidney injury and may be an accurate predictable biomarker for CKD. As a transmembrane molecule, KIM-1 is undetectable in the tubular cells of patients with healthy kidneys.¹³ In patients with damaged kidney where tubular epithelial cells have remarkable differentiation and proliferation, high concentrations of KIM-1 can be detected in the urine and plasma samples.⁴ In the early detection of kidney disease, the serum creatinine has low accuracy and validity thus appropriate serum markers are crucial for the prognosis and diagnosis of CKD.¹⁴ Given the above-mentioned explanations, this study was conducted to evaluate NGAL, KIM-1, and L-FABP serum levels in CKD patients.

Materials and Methods

This cross-sectional study was performed in the Department of Biochemistry, Shahid Sadoughi University of Medical Sciences. The study population consisted of 42 CKD patients from Imam Khomeini Hospital (Kangan) and healthy persons who were matched in terms of gender and age. The exclusion criteria were urinary tract infection, hypertension, heart failure, acute infectious diseases, hematuria, recent severe exercise history, and pregnancy. Written informed consent was taken from all participants. Afterward, 5 cc of venous blood was collected in tubes containing ethylenediaminetetraacetic acid from all subjects in the morning and after 12 hours of fasting. To isolate the serum, blood samples were centrifuged at 3000 rpm for 20 minutes and then stored at -20°C until use.

ELISA and Jaffe's Methods

The Commercial Enzyme-Linked Immunosorbent Assay kit (Bioassay Technology Laboratory, China) was used to detect NGAL (Cat.No E5774Hu, Standard Curve

Range: 0.1 ng/mL – 40 ng/mL, sensitivity: 0.058 ng/mL, intra-assay: CV<8%, inter-assay: CV<10%), KIM-1 (Cat No E1099Hu, standard range: 0.05 ng/mL–10 ng/mL, sensitivity: 0.01 ng/mL, intra-assay: CV<8%, inter-assay: CV<10%), and L-FABP (Cat No E2159Hu, sensitivity: 4.56 ng/L, standard range: 8 ng/L–1800 ng/L, intra-assay: CV<8%, inter-assay: CV<10%) serum levels. Next, the serum creatinine level was measured by the Jaffe's method with 0.2-15 mg/dL (Pars Azmun, Iran).

Sample Size

The sample size was calculated as 42 by G*Power 3.1.9.2. (University of Kiel, Kiel, Germany). Using the *t* test correlation, the point biserial model for the effect size of 0.6 with the coefficient of determination p^2 with the power of 95% and type I error rate of 0.05 was applied.

Statistical Analysis

The statistical analysis was performed using SPSS, version 18. Continuous variables with normal and non-normal distributions were described by the mean and median (quartile), respectively. In addition, all categorical variables were shown as frequency and percentages. Data with a normal distribution were compared with Student's *t* test, and the Mann-Whitney U test was applied to compare the groups in case of a non-normal distribution. Further, Pearson and Spearman correlation coefficients were employed to assess parametric and non-parametric data, respectively. For all tests, the significant level was considered as $P < 0.05$.

Results

NGAL, KIM-1, and L-FABP values in serum samples from patients and the control group were determined using relevant ELISA kits, and then the concentrations of these molecules were detected from the standard curve. As shown in Figure 1, the mean serum levels of NGAL (449.37 ± 195 vs. 58.57 ± 32.2 ng/mL, $P = 0.001$), KIM-1 (7.44 ± 2.9 vs. 2.33 ± 1.9 ng/mL, $P = 0.001$), and L-FABP (1088.56 ± 685 vs. 162.34 ± 112 ng/L, $P = 0.001$) were significantly higher in CKD in comparison with the control group. In addition, serum creatinine levels were higher in patients (6.26 ± 2.41 mg/dL) compared to healthy subjects (0.9 ± 0.2 mg/dL), the related data are displayed in Figure 2 ($P = 0.001$).

Based on the results, no significant correlation was found between serum creatinine levels and the three measured factors neither in patients nor controls. Conversely, significant positive correlations were observed among the serum levels of NGAL, L-FABP, and KIM-1 (Table 1).

In addition, receiver operating characteristic curve analysis was employed to address the diagnostic values of NGAL, L-FABP, and KIM-1 circulating levels for CKD. The analysis revealed that the area under the curve was 0.89, 0.86, and 0.88 for NGAL, L-FABP, and KIM-1 ($P < 0.001$), respectively (Figure 3).

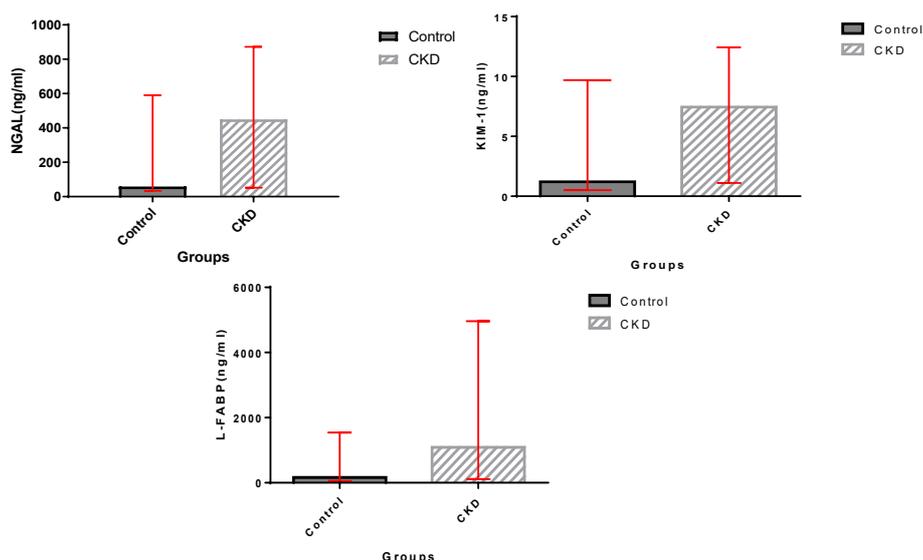


Figure 1. Levels of NGAL, KIM-1, and L-FABP in Both Patient and Control Groups.
 Note. Neutrophil gelatinase-associated lipocalin (NGAL); KIM-1: Kidney injury molecule-1; L-FABP: Liver-type fatty acid-binding protein; CKD: Chronic kidney disease. The values of all three molecules were significantly higher in the CKD group compared to the control group.

Note. ROC: Receiver operating characteristic curve; AUC: Area under the curve. The AUC was 0.86 for L-FABP with a sensitivity of 83.3% and specificity of 78.3%. In addition, it was 0.88 for KIM-1 with a sensitivity of 85.7% and specificity of 78.6%. Moreover, this value was 0.89 for NGAL with a sensitivity of 87.6% and specificity of 79.3%.

Discussion

CKD is characterized by renal injury and a decline in renal function (eGFR) that lasts for 3 months or more.¹⁴ The rate of CKD is increasing while the applied health care resources are inappropriate.⁹ One of the main concerns in CKD patients is the early detection of renal impairment. Generally, serum creatinine concentration is used as an indicator of renal function but this factor reflects reduced

glomerular filtration in the final stage of the disease, which limits its ability to diagnose renal dysfunction.¹⁵ Three NGAL, L-FABP, and KIM-1 molecules have recently been identified as the marker for determining renal function impairments and reflect the ongoing process of renal injury.¹⁶

In this study, the diagnostic value of these three factors was compared to evaluate kidney function in CKD patients. It was found that the serum levels of NGAL in CKD patients were significantly higher compared to their healthy peers. The results of this study are in line with those of Ezenwaka and Malyszko, which showed that the serum and urinary levels of NGAL significantly increased in CKD patients.^{17,18} Therefore, NGAL may be used as a diagnostic parameter in patients with acute kidney damage. Based on the findings of the present study, there was no significant association between the serum levels of NGAL and creatinine. However, in two studies conducted by Ezenwaka et al and Avci et al, along with the elevation of NGAL, serum creatinine concentration was associated with serum NGAL levels.^{17,19} This contradiction in the results of the present study with those of the above-mentioned studies may be due to differences in the patient population. In the present study, some CKD patients had other problems, including cardiovascular diseases and diabetes. On the other hand, in the recent two studies, half of the patients underwent kidney transplantation which may have significantly affected creatinine renal excretion. Interestingly, NGAL has attracted remarkable attention since new results suggested NGAL as a prognostic marker in kidney disease. A clinical study reported that the serum levels of NGAL were significantly associated with kidney function and acute kidney injury.¹⁸ In addition, the *in vitro* studies of renal failure models revealed that the increased

Table 1. Pearson Correlation Between the NGAL, L-FABP, and KIM-1 Measured in the CKD and Control Groups

	Variables	P Values	Correlation Coefficient
CKD Patients	Cr/NGAL	0.597	-0.084
	Cr/KIM-1	0.545	-0.096
	Cr/L-FABP	0.246	-0.183
	NGAL/KIM-1	0.001*	0.961
	NGAL/L-FABP	0.001*	0.868
	L-FABP/KIM-1	0.001*	0.907
	Control	Cr/NGAL	0.698
Cr/KIM-1		0.556	0.093
Cr/L-FABP		0.637	0.075
NGAL/KIM-1		0.001*	0.784
NGAL/L-FABP		0.001*	0.809
L-FABP/KIM-1		0.001*	0.828

Abbreviation: NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid binding protein; KIM-1, kidney injury molecule-1; CKD, chronic kidney disease

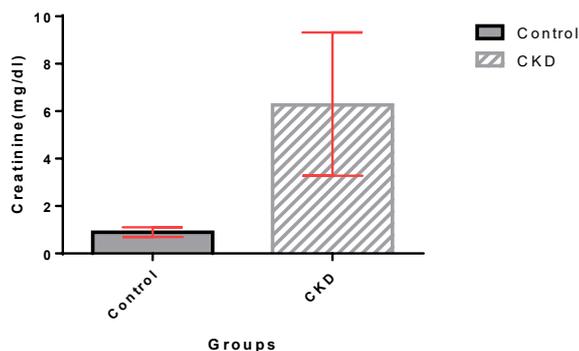


Figure 2. Comparison of Serum Creatinine Concentrations in CKD and the Control Group.

Note. CKD: Chronic kidney disease

mRNA expression of NGAL has beneficial effects in renal failure patients.^{18,20}

It was also found that serum KIM-1 concentrations were significantly higher in the CKD group compared to healthy controls. These results are in line with those of Lentini et al who argued that KIM-1 could be used as a marker for detecting CKD.²¹ Additionally, in a cohort study by Viau et al, it was shown that the serum level of KIM-1 could be a key factor for the diagnosis and progression of CKD.¹⁶ However, serum KIM-1 was not correlated with creatinine levels in the present study, which corroborates with the findings of Zulu et al indicating no significant correlation between KIM-1 and creatinine.²² Therefore, the measurement of KIM-1 levels can be used as an independent marker for the diagnosis of CKD.

Moreover, our results showed that the serum concentration of L-FABP was significantly higher in the CKD group compared to the control group. Likewise, Waikar et al found that L-FABP was associated with the progression of CKD, and in a study conducted by Nakagawa et al, L-FABP was noted to predict CKD progression.^{23,24} Similarly, Waanders et al demonstrated

that NGAL and L-FABP were related to CKDs.²⁵ The results of all three studies are consistent with those of our study. On the other hand, the serum levels of L-FABP represented no significant correlation with serum creatinine concentrations in the present study. Contrary to the results of our study, Abe et al concluded that L-FABP concentrations in the urine had a significant relationship with urinary creatinine concentrations and could be a marker for the detection of CKD.²⁶ In addition to measuring the urinary values of these two factors, patients in the above-mentioned study were non-diabetic while the serum levels of L-FABP and creatinine were measured and diabetic patients were included in this study. Considering that diabetes can affect the balance of creatinine clearance in the kidneys and serum creatinine concentrations, this may partly explain the difference in the outcomes of these two studies.

The findings of the present study revealed that the serum levels of NGAL, L-FABP, and KIM-1 were significantly higher in CKD patients as compared to healthy controls. Given that NGAL, L-FABP, and KIM-1 had a significant positive correlation with each other, these factors together can provide an accurate indicator for the diagnosis of CKD.

Conclusion

In general, the serum levels of NGAL, L-FABP, KIM-1, and creatinine were significantly higher in patients with CKD compared to the control group. Therefore, each of these four factors can be used as an independent marker for the diagnosis of CKD. The serum concentrations of NGAL, L-FABP, and KIM-1 were also associated with each other, indicating the value of the serum levels of these markers together for the diagnosis of CKD. It can be concluded that increasing the concentration of the serum levels of each of these three molecules predicts an increase in the concentration of two other molecules.

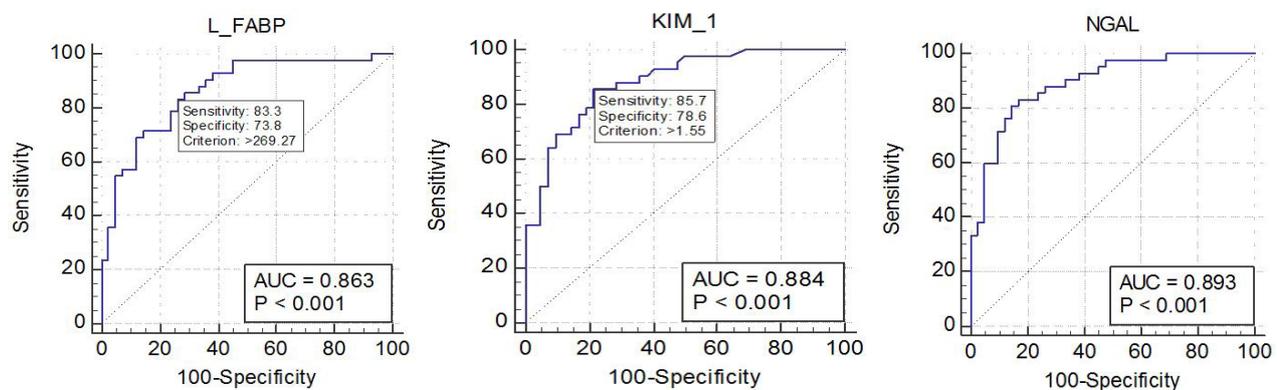


Figure 3. ROC Curve Analysis for NGAL, KIM-1 and L-FABP Serum Levels.

Note. ROC: Receiver operating characteristic curve; AUC: Area under the curve. The AUC was 0.86 for L-FABP with a sensitivity of 83.3% and specificity of 78.3%. In addition, it was 0.88 for KIM-1 with a sensitivity of 85.7% and specificity of 78.6%. Moreover, this value was 0.89 for NGAL with a sensitivity of 87.6% and specificity of 79.3%.

Ethical Approval

The study was approved by the Local Ethics Committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1395.167), and all participants gave informed written consent according to the 1964 Declaration of Helsinki.

Conflict of Interest Disclosure

There is no conflict of interests regarding the publication of this article.

Authors' Contributions

TO conducted the experiment and measurements and drafted the manuscript. In addition, HR designed and performed the experiments, analyzed the data, and wrote the manuscript. Furthermore, JZR designed and directed the project and wrote the paper with input from all authors. Finally, all authors discussed the results and commented on the manuscript.

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