Saudi J Kidney Dis Transpl 2020;31(2):360-367

Saudi Journal of Kidney Diseases and Transplantation

Original Article

Novel Biomarkers of Acute Kidney Injury Following Living Donor Liver Transplantation

Mohamed Sherif Mogawer[1], Mostafa Abd El-Rahman El-Shazly[2], Ahmed Yamany Ali[1], Ahmed Mohamed Abd El-Ghany[1], Samah Abd Elhamid[3]

Departments of [1]Internal Medicine, [2]General Surgery and Liver Transplantation and [3]Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Giza, Cairo, Egypt

ABSTRACT. Urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) and renalase were recently studied for their potential role in the early detection of acute kidney injury (AKI) in patients with cirrhosis. Our study was conducted on 50 patients with end-stage liver disease undergoing living donor liver transplantation. The patients were divided into two groups: Group I contained 23 patients with AKI who had undergone liver transplantation and Group II included 27 non-AKI patients who had undergone liver transplantation. Serum renalase and NGAL levels were measured by ELISA; renalase was measured on day 1, day 7, and three months after liver transplantation. NGAL was measured on day 1 postliver transplantation. There was an improvement in liver function, kidney functions, hemoglobin level, platelet count, and C-reactive protein levels in patients at three months posttransplantation when compared to day 1, day 3, and day 7 ($P < 0.01$). Comparison of the renalase level at day 1, day 7, and three months showed that there was a highly significant decline at three months in the AKI group compared to the non-AKI group ($P < 0.01$). Regarding the NGAL level at day 1, there was no significant difference between the AKI and non-AKI groups ($P > 0.05$). The receiver operating characteristic curve for the renalase biomarker showed a borderline significant change between the AKI and non-AKI groups at day 1 [area under the curve (AUC): 0.54, $P = 0.08$], day 7 (AUC: 0.605, $P = 0.08$), and three months (AUC: 0.605, $P = 0.08$). However, the NGAL biomarker level was not significantly different between the AKI and non-AKI groups. Our study suggests that renalase showed a better predictive value and a higher accuracy in identifying postliver transplantation patients with AKI than NGAL.

Correspondence to:

Dr. Ahmed Yamany Ali,
Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt.
E-mail: dr.yamany79@yahoo.com

Introduction

Cirrhosis is the eight leading causes of death in the United States.[1] Patients with cirrhosis are typically candidates for liver transplantation once complications of portal hyperten-

sion or hepatocellular insufficiency develop. Transplantation evaluation is typically initiated when the patient has a model of end-stage liver disease (MELD) score above 10.

Renal dysfunction is a common and serious complication in patients with advanced liver disease. For many years, serum creatinine (Cr) has been used as a biomarker of renal function in patients with acute renal failure; however, this biomarker has many limitations in clinical practice since it is influenced by race, age, gender, and body weight. In addition, patients with liver cirrhosis frequently develop muscle wasting with decreased formation of Cr from creatine; such patients develop renal tubular loss of Cr.[2]

Recently, most experts have agreed on the potential role of new serum and urinary biomarkers in the differential diagnosis of different types of acute kidney injury (AKI) in patients with cirrhosis; markers of tubular injury, such as neutrophil gelatinase-associated lipocalin (NGAL), renalase, kidney injury molecule-1, interleukin-18, and liver fatty acid-binding protein, have been shown in recent years to be useful in the differential diagnosis of AKI in patients with liver cirrhosis.[3-5]

AKI is common after liver transplantation, affecting 25%–50% of recipients.[6] It is well known that postoperative AKI has an impact on both mortality and the development of chronic renal failure in liver transplant recipients.[7]

Interestingly, most patients who survive up to six months posttransplantation develop renal impairment, and such patients are at a greater risk of cardiovascular events, hospitalization, and mortality than recipients with preserved renal function.[8]

In our study, we tried to elucidate the role of novel biomarkers such as renalase and NGAL in the early prediction of AKI in the perioperative period following living donor liver transplantation (LDLT). Furthermore, whether early detection of AKI following liver transplantation is related to calcineurin inhibitors (CNIs) and whether therapeutic strategies can be adjusted to minimize their nephrotoxic effects were also assessed.

Subjects and Methods

Subjects

The present study was conducted on 50 patients with end-stage liver disease undergoing LDLT. The patients were divided into two groups: Group I contained 23 AKI patients following liver transplantation, whereas Group II included 27 non-AKI patients following liver transplantation. Patients were recruited from El-Manial Specialized Hospital of Cairo University in the period from March 2014 to July 2017, and they were followed up for three months.

Methods

All patients provided a thorough history and were subjected to a detailed physical examination, providing data on age, gender, comorbid diseases (diabetes, hypertension, and dyslipidemia), concomitant medications (renin–angiotensin–aldosterone system blockers and diuretics), and body mass index (BMI), before surgery. Patients were also evaluated using the Child–Pugh and MELD scores. Abdominal Doppler ultrasonography was performed. Intraoperative evaluation of patients included complete blood count (CBC), liver function tests (LFTs), prothrombin concentration, kidney function tests (KFTs), electrolytes, and abdominal Doppler ultrasonography. Postoperative evaluation included daily examination of vital signs, drains, and fluid balance. Daily measurement of CBC, LFT, and KFT was performed for seven days and at three months. CNI levels were checked regularly. Doppler ultrasound was performed to assess graft homogeneity, biliary complications, the portal trunk, and hepatic vessels. Specific laboratory investigations, including immunoassay of serum NGAL and serum renalase, were performed at 6 h, one week, and three months after transplantation.

Serum Neutrophil Gelatinase-associated Lipocalin and Renalase

Principle of the assay

The microtiter plate provided in the kit had been precoated with an antibody specific to serum NGAL or serum renalase. Standards or samples were added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for serum NGAL or serum renalase. Avidin-conjugated horseradish peroxidase was added to each microplate well and incubated. Then, a substrate solution was added to each well. Only wells that contained biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme–substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of the markers in the samples was then determined by comparing the optical density of the samples to the standard curve.

Detection range

Serum NGAL

Serum NGAL levels ranged from 156 to 5000 pg/mL. The standard curve concentrations used for the ELISA were 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, and 156 pg/mL.

Serum renalase

Serum renalase levels ranged from 0.78 to 50 ng/mL. The standard curve concentrations used for the ELISA ranged from 0 to 50 ng/mL.

Calculation of results

The mean absorbance was calculated for each set of duplicate standards, controls, and samples, and the average zero standard optical density was subtracted. The standard curve was plotted using Sigma plot software (Scientific Data Management Company, UK), with the standard concentration on the x-axis and absorbance on the y-axis. The best-fit straight line was drawn through the standard points.

Statistical Methods

Data were coded and entered using the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, Ill, USA). Data were summarized using the mean and standard deviation for the quantitative variables. Comparisons between groups were performed using analysis of variance with a multiple comparison *post hoc* test for quantitative variables. $P < 0.05$ was considered statistically significant. The receiver operating characteristic (ROC) curve was used to show the sensitivity and specificity and to determine the optimal cutoff value. Correlations between measured parameters and laboratory data were analyzed using the Spearman's correlation coefficient.

Results

This prospective study was conducted on 50 patients who had undergone LDLT. Their ages ranged from 20 to 64 years, with a mean value of 49.28 ± 9.28 years. There were 36 males (72%) and 14 females (28%). The selected patients were classified according to whether they developed AKI: the AKI group (Group I) contained 23 patients who developed AKI and the non-AKI group (Group II) included 27 patients without AKI. Thirteen patients who developed AKI died three months after transplantation.

By comparing different laboratory data at three months postliver transplant to laboratory data on days 1, 3, and 7, a significant difference was observed in most laboratory data, with improvements in liver function, kidney function, hemoglobin (Hb) level, platelet count, and C-reactive protein (CRP) level at three months posttransplantation, when compared to days 1, 3, and 7 (Table 1).

By comparing the mean renalase level between the AKI and non-AKI groups at day 1, day 7, and three months, a highly significant decline was observed at three months in the AKI group compared to the non-AKI group ($P = 0.003$) (Table 2).

There was no statistically significant difference

Table 1. Statistical comparison of the mean value of different laboratory data at day 1, day 3, day 7, and 3 months in all patients.

Parameter	Day 1	Day 3	Day 7	3 months	*P*
Liver biochemical profile					
Total bilirubin (mg/dL)	5.33±2.40	3.95±2.69	4.40±4.14	1.63±1.23	0.001
Direct bilirubin (mg/dL)	2.80±1.15	2.60±2.18	3.09±3.22	0.99±1.05	0.001
AST (U/L)	375.05±230.01	217.22±274.58	83.04±58.34	56.83±51.8	0.001
ALT (U/L)	296.08±210.55	240.76±304.92	115.20±77.64	54.40±48.43	0.001
Albumin (g/dL)	3.20±0.22	2.73±0.38	2.70±0.55	3.82±0.35	0.002
Total protein (g/dL)	4.70±1.22	4.45±0.75	4.44±0.86	4.80±0.87	0.08
INR	2.22±0.71	1.61±0.43	1.29±0.29	1.13±0.09	0.001
GGT (U/L)	45.40±19.02	65.20±37.61	110.90±0.29	85.02±65.80	0.001
ALP (U/L)	49.50±16.05	64.04±42.95	97.68±55.92	101.01±112.55	0.001
Kidney profile					
Creatinine (mg/dL)	1.36±0.60	1.29±0.71	1.33±0.78	0.94±0.23	0.001
Urea (mg/dL)	44.88±19.02	80.50±42.58	89.80±45.31	37.37±13.91	0.002
Electrolytes					
Sodium (mEq/L)	136.05±4.80	136.84±5.39	131.74±19.8	138.59±3.83	0.25
Potassium (mEq/L)	4.02±1.10	4.17±0.57	3.85±0.69	4.23±0.62	0.12
Uric acid (mg/dL)	3.90±1.12	4.01±1.36	4.2±1.4	4.07±1.59	0.14
Complete blood count					
Hemoglobin (g/dL)	8.90±2.50	8.50±0.92	9.01±3.76	11.87±1.48	0.001
Total leukocyte count	8.72±2.05	7.12±4.36	9.13±60	5.77±1.88	0.09
Platelets	88.60±30.04	66.42±34.29	84.72±49.77	181.40±59.04	0.001
CRP (mg/L)	6.40±2.05	14.04±3.97	14.80±9.50	3.85±0.50	0.001
Renalase (ng/mL)	1.4±0.3		1.25±0.4	1.03±0.4	0.001
NGAL (ng/mL)	1.8±0.35				

P <0.05 is significant, *P* <0.01 is highly significant. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalized ratio, GGT: Gamma-glutamate transpeptidase, ALP: Alkaline phosphatase, CRP: C-reactive protein, NGAL: Neutrophil gelatinase-associated lipocalin.

Table 2. Comparison of serum renalase and neutrophil gelatinase-associated lipocalin levels between groups with and without acute kidney injury by ANOVA.

	Non-AKI group	AKI group	*P*
Renalase (ng/mL) day 1	1.18±0.48	1.32±0.45	0.003 HS
Renalase (ng/mL) day 7	1.06±0.38	1.18±0.35	
Renalase (ng/mL) 3 months	1.02±0.32	0.90±0.27	
NGAL (ng/mL)	1.7±0.4	1.9±0.4	0.44

Two-way ANOVA ($P<0.05$ is significant, and $P<0.01$ is highly significant). AKI: Acute kidney injury, NGAL: Neutrophil gelatinase-associated lipocalin.

in tacrolimus levels at day 3 and at day 7 postoperatively between non-AKI and AKI patients ($P>0.05$).

There was a statistically significant positive correlation between renalase and aspartate aminotransferase (AST) levels at day 7 (r = 0.49, P = 0.001). A statistically significant positive correlation of renalase with urea, Cr, TLC, and CRP was also observed at three months posttransplantation in the AKI patients (r = 0.64, 0.74, 0.47, 0.44, respectively, $P<0.001$).

A statistically significant inverse correlation between the NGAL biomarker with Cr and urea was observed at day 1 (r = −0.7, −0.66, $P<0.001$, 0.001, respectively). However, comparison of the NGAL level between the AKI and non-AKI groups at day 1 showed no significant difference ($P>0.05$) (Table 2).

ROC curve analysis of markers was conducted in this study, and renalase levels showed a borderline significant change between the AKI and non-AKI groups at day 1 [area under the curve (AUC): 0.54, P = 0.08, 78% sensitivity and 55% specificity at a cutoff level of 0.85], day 7 (AUC: 0.605, P = 0.08, 86% sensitivity and 61% specificity at a cutoff level of 0.74), and three months (AUC: 0.605, P = 0.08, 86% sensitivity and 61% specificity at a cutoff level of 0.73) (Figure 1). In addition, the NGAL level did not show a significant change between the AKI and non-AKI groups (AUC: 0.52, P = 0.44, 60% sensitivity and 67% specificity at a cutoff level of 1.85).

Discussion

Owing to the delayed rise in serum Cr concentration in AKI, a new class of novel biomarkers has shown a significant promise in

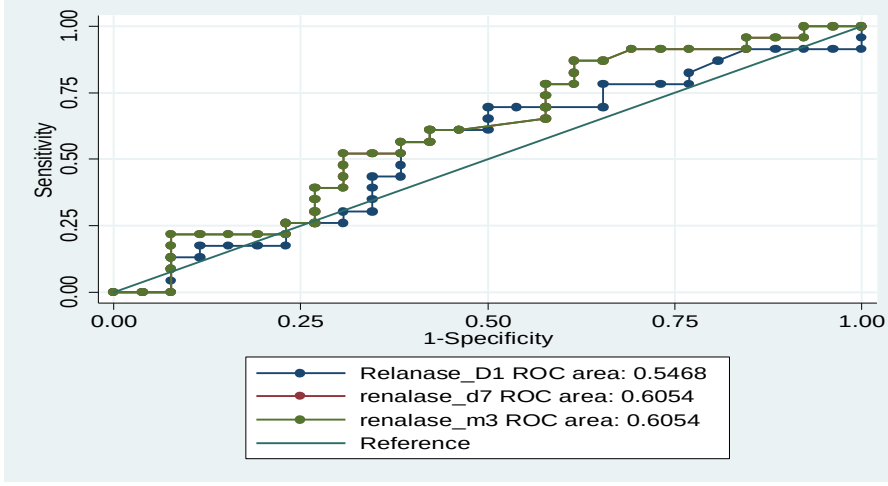

Figure 1. ROC curve analysis showing the diagnostic power of renalase in the prediction of acute kidney injury.

D7 and M3 are overlapping. ROC: Receiver operating characteristic, D1: Day 1, D3: Day 3, M3: Month 3.

improving the prediction of AKI. These biomarkers include NGAL and renalase.[9] In this study, we tried to determine whether NGAL or renalase can be used as potential biomarkers of AKI and whether these biomarkers can be used to assess renal injury related to calcineurin inhibitors, which may allow the adjustment of therapeutic strategies to minimize their nephrotoxic effects. The current prospective study was conducted on 50 patients who had undergone living donor liver transplantation at El-Manial Specialized University Hospital. The patients were divided into two groups: the AKI group (Group I) which included 23 patients who developed Stage I AKI after day 1, which included seven patients who remained at Stage I AKI, one patient who progressed to Stage 3 AKI on day 7 posttransplantation, and two patients who recovered and the non-AKI group (Group II) included 27 patients without AKI. The mean age of the studied groups was 49.28 ± 9.28 years. They were 36 males (72%) and 14 females (28%), with a mean BMI of 25.7; four patients (8%) were hypertensive, seven (14%) had diabetes, and 42 (84%) had hepatitis C-related chronic liver disease. By comparing different laboratory data at the 3rd month to laboratory data with data on days 1, 3, and 7, a significant improvement of most laboratory data reflecting graft function, including AST, ALT, bilirubin, albumin, INR, platelet count, and Hb, was observed. This result agrees with that of Moon and Lee,[10] who found that liver biochemistries steadily improve following liver transplantation surgery as ischemia and reperfusion injury resolve. Our study showed a statistically significant increase in mean serum Cr at days 1, 3, and 7 compared to three months postoperatively. This result matched the report of Nastos et al,[11] who demonstrated that acute renal dysfunction presents often after liver transplantation, and this may be attributed to hepatic ischemia–reperfusion injury and oxidative stress-causing renal tubular cell injury. We showed in our study a statistically significant positive correlation between renalase and serum Cr at day 1 and at the 3rd month. These data were in agreement with the data of Gaber and El-Attar,[12] who conducted their study on 80 Egyptian patients with diabetes and found that patients with higher serum Cr levels had significantly higher blood pressure, albumin-creatinine ratio, and serum renalase levels. There are considerable discrepancies regarding renalase level and its activity in patients with AKI, patients with chronic kidney disease, and patients on renal replacement therapy. Desir[13] showed a significant reduction in renalase level in chronic kidney disease and hemodialysis patients. This situation may be explained by different methods of renalase level estimation and the different antibodies used in different studies. Data in the 3rd month displayed a statistically significant positive correlation between renalase and the total leukocyte count and CRP level. This finding matches the opinion of Zhao et al,[14] who reported that renalase exhibited an anti-inflammatory effect through suppressing renal-infiltrated macrophages. To our knowledge, this might be the first study to demonstrate that there is a statistically significant positive correlation between renalase and AST at day 1 and day 7 and between renalase and direct bilirubin at day 1. This result might be attributed to an early inflammatory process occurring with ischemia–reperfusion injury and to the anti-inflammatory effect of renalase. In our study, we performed a statistical comparison of mean renalase levels at day 1, day 7, and the 3rd month postoperatively, and we found a statistically significant decline in mean renalase level at seven days and three months compared to day 1. This finding agrees with that of Wang et al,[15] who reported in their study that the renalase level was reduced following acute renal ischemia. This observation can also be explained by the study conducted by Lee et al,[16] who suggested that renalase may protect against inflammatory tissue injury and that its level may decrease after the resolution of inflammation. More recent support was provided by Ibrahim et al,[17] who stated that renalase might represent an early and a more sensitive biomarker of ischemic renal injury than serum Cr or other biomarkers, such as NGAL, in patients undergoing cardiac

surgery. In our study, we found no statistically significant correlation between tacrolimus levels and the development of AKI, which does not agree with the findings of Trotter and Levy,[18] who concluded that the early use of calcineurin inhibitor postliver transplantation is associated with impaired renal function. A statistically significant inverse correlation between NGAL and Cr and urea was observed on day 1 (r = −0.7, −0.66, P <0.001, 0.001, respectively). No statistically significant difference was noted in NGAL level between the AKI and non-AKI groups (P >0.05). This finding is in contrast to that of Gavrić and Kališnik,[19] who stated that NGAL is a good marker that can detect early renal damage after cardiac surgery in adult patients. Furthermore, it falls within the interest of our study to conduct ROC curve analyses for both biomarkers. The renalase biomarker showed a better predictive value and a higher accuracy in identifying patients with AKI postliver transplantation than the NGAL biomarker; contradictory results may be due to uncertainty regarding whether renalase is a renal-specific biomarker since it is released from different sources.

Study Limitations

Further studies on larger populations are required to validate our reports and to evaluate the potential utility of renalase measurement in the early prediction and overall progression of AKI postliver transplantation.

Acknowledgments

We would like to thank El-Manial Specialized University Hospital employees for their assistance and guidance in this research. We also thank our patients for their willing participation in our research.

Declaration of Ethics

This study was approved by the Review Board of Kasr Alainy Hospital. Oral and written informed consent was obtained from all patients according to the Helsinki guidelines of research ethics.

Conflict of interest: None declared.

References

1. Egerod Israelsen M, Gluud LL, Krag A. Acute kidney injury and hepatorenal syndrome in cirrhosis. J Gastroenterol Hepatol 2015;30: 236-43.
2. Caregaro L, Menon F, Angeli P, et al. Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. Arch Intern Med 1994;154:201-5.
3. Fagundes C, Pépin MN, Guevara M, et al. Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. J Hepatol 2012;57:267-73.
4. Belcher JM, Sanyal AJ, Peixoto AJ, et al. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. Hepatology 2014;60:622-32.
5. Wybraniec MT, Bożentowicz-Wikarek M, Chudek J, Mizia-Stec K. Urinary renalase concentration in patients with preserved kidney function undergoing coronary angiography. Nephrology (Carlton) 2018;23:133-8.
6. Kırnap M, Colak T, Baskın E, et al. Acute renal injury in liver transplant patients and its effect on patient survival. Exp Clin Transplant 2014;12 Suppl 1:156-8.
7. Hilmi IA, Damian D, Al-Khafaji A, et al. Acute kidney injury following orthotopic liver transplantation: Incidence, risk factors, and effects on patient and graft outcomes. Br J Anaesth 2015;114:919-26.
8. Nadim MK, Genyk YS, Tokin C, et al. Impact of the etiology of acute kidney injury on outcomes following liver transplantation: Acute tubular necrosis versus hepatorenal syndrome. Liver Transpl 2012;18:539-48.
9. Liu S, Che M, Xue S, et al. Urinary L-FABP and its combination with urinary NGAL in early diagnosis of acute kidney injury after cardiac surgery in adult patients. Biomarkers 2013;18:95-101.
10. Moon DB, Lee SG. Liver transplantation. Gut Liver 2009;3:145-65.
11. Nastos C, Kalimeris K, Papoutsidakis N, et al. Global consequences of liver ischemia/ reperfusion injury. Oxid Med Cell Longev

2014;2014:906965.
12. Gaber EW, El-Attar HA. The relationship between dopamine and renalase in type 2 diabetic patients with and without diabetic nephropathy. Br J Diabetes Vasc Dis 2013; 13:130-7.
13. Desir GV. Renalase deficiency in chronic kidney disease, and its contribution to hypertension and cardiovascular disease. Curr Opin Nephrol Hypertens 2008;17:181-5.5.
14. Zhao B, Zhao Q, Li J, Xing T, Wang F, Wang N. Renalase protects against contrast-induced nephropathy in Sprague-Dawley rats. PLoS One 2015;10:e0116583.
15. Wang L, Velazquez H, Moeckel G, et al. Renalase prevents AKI independent of amine oxidase activity. J Am Soc Nephrol 2014; 25:1226-35.5.
16. Lee HT, Kim JY, Kim M, et al. Renalase protects against ischemic AKI. J Am Soc Nephrol 2013;24:445-55.
17. Ibrahim IA, Sayed HA, Mohammed AA. Plasma renalase as a biomarker of acute kidney injury after cardiac surgery. Egypt J Intern Med 2016;28:91-8.
18. Trotter JF, Levy G. Sotrastaurin in liver transplantation: Has it had a fair trial? Am J Transplant 2015;15:1137-8.
19. Gavrić A, Kališnik JM. Novel biomarkers for early diagnosis of acute kidney injury after cardiac surgery in adults. Kardiochir Torakochirurgia Pol 2016;13:31-8.

Date of manuscript receipt: *24 September 2018.*
Date of revised copy receipt: *28 November 2018.*
Date of final acceptance: *2 December 2018.*