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Original article

Urinary neutrophil gelatinase associated lipocalin as an early marker of acute kidney injury in the recipient after liver transplantation

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ABSTRACT

Background: Urinary NGAL is a novel biomarker that is rapidly released after AKI.
Purpose: To study the value of urinary NGAL/creatinine ratio in predicting AKI in the recipient of LDLT.
Methods: A total of 30 recipients of LDLT were included in a prospective, randomized, two center study. All patients were subjected to a measurement of urinary NGAL/creatinine ratio and the serum creatinine at fashioned time intervals to assess which can predict early renal impairment; accordingly, these data were applied on mortality and hospital stay.
Results: Urinary NGAL/creatinine ratio was significant in detecting renal impairment as early as 3, 18, and 24 h post induction of anesthesia ($p = 0.001$ for all), while it was not significant preoperatively ($p = 0.817$). Serum creatinine was not significant during the 1st and 2nd days ($p = 0.748$ and 0.157 respectively), but began to be significant during the 3rd and 4th days ($p < 0.001$ for both). Urinary NGAL/creatinine ratio was correlated with ICU stay ($r = 0.758$ with $p < 0.001$) and mortality during 3, 18, and 24 h postoperative ($p < 0.001$, <0.001 and 0.005 respectively). The most sensitive in detecting mortality was urinary NGAL/creatinine ratio after 3 h of the operation with a sensitivity of 100% and a specificity of 95.5%.
Conclusion: Urinary NGAL/creatinine ratio may be used as a test for the early prediction of adverse outcome of LDLT recipient patients at ICU admission.

1. Introduction

Acute kidney injury (AKI) after liver transplantation (LT) is a frequent complication and is associated with a substantial increase in morbidity, length of stay, and mortality. The cause of AKI after LT is multifactorial and multiple insults and risk factors are required for AKI to progress to renal failure. Pre-operative renal dysfunction, hepato-renal syndrome, caval cross-clamping with renal vein outflow obstruction, Intra- and post-operative hypotension, high vasopressor requirements, large volume transfusions, and the post-operative use of calcineurin inhibitors all contribute to renal injury that may lead to AKI [1,2].

Serum creatinine, the commonly used marker for renal injury, is slow and insensitive. It is a marker of function and not injury, and is unable to detect subtle injuries or delineate a single cause of renal injury. It is diluted by large-volume transfusions and may take days after injury to increase. Therefore, an increase of serum creatinine reflects the sum of injuries in the preceding days and cannot identify the effect of a single renal insult or subtle differences between groups of patients [3].

NGAL is a small, 25-kDa protein of the lipocalin family. It was discovered through a genome-wide analysis of kidney genes that are induced in response to AKI. It was among the top upregulated genes in damaged kidneys and fulfilled the criteria of a promising biomarker of tubular damage since it was a secreted tubular protein that entered both urine and serum rapidly after the onset of AKI. Some physicians prefer to name it troponin of the kidney, as it is a novel biomarker that is detectable very early after renal injury [2].

It is upregulated in renal tubular cells within minutes after ischemia reperfusion injury and secreted in urine [4]. Studies on patients undergoing cardiopulmonary bypass revealed a marked

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increase of plasma and urinary NGAL levels as early as 2 h after surgery in patients that developed AKI. This increase in urinary NGAL preceded the increase in serum creatinine by 24–48 h. All these factors made NGAL a good predictor of AKI in many clinical scenarios, such as in septic shock or with contrast induced nephropathy. Plasma NGAL has recently been described as a good predictor of AKI after LT, but urinary NGAL after LT has not been extensively studied to date [5].

The aim of the current study is to evaluate the urinary NGAL/creatinine ratio after LT and to determine its ability to detect acute structural injury and predict clinical AKI after LT compared with traditional serum creatinine.

2. Patients and methods

2.1. Patients

Thirty patients diagnosed with end stage liver disease who were scheduled to undergo LT at Cairo University hospital and El Sahel Teaching hospital, Cairo, Egypt were enrolled prospectively during the period from January 2013 to June 2015. The study was approved by the institution review board at Cairo University and El Sahel Teaching hospitals. Informed consent was obtained from all patients participating in the study. If patients were aged less than 21 years old, then consent was obtained from their legal guardian.

2.1.1. Inclusion criteria

1) Age $\geqslant$18 and <65 years old, 2) All patients are expected to stay in the ICU for 5 days (according to Japanese protocol all the recipients must lay down on their backs for 5 days to allow fixation of the graft) [6], 3) All patients without pre-operative renal impairment or failure.

2.1.2. Exclusion criteria

included Pregnant patients, those with preexisting medical condition with a life expectancy of less than 3 months, those requiring a combined liver kidney transplantation, and those with a history of previous LT.

Patients who met the inclusion criteria and did not meet the exclusion criteria were studied and included into the study, and they were followed up until the day of discharge from ICU.

The studied patients were subdivided into two groups according to the development of renal impairment during the ICU stay (according to RIFLE criteria). Group I: patients with renal impairment, and Group II: patients without renal impairment. The RIFLE classification defines three grades of severity of AKI (Risk, Injury and Failure) based on changes of serum creatinine and urine output or both and two clinical outcomes (Loss, End-stage renal disease). Risk is defined as a serum creatinine elevation of at least 50%. Injury is considered when the serum creatinine level increased by 100% and failure is considered when the creatinine level is 300% higher than the baseline [7,8].

2.2. Evaluation of patients

All included patients were subjected to the following:

2.2.1. Full clinical evaluation

Including history and physical examination with a special emphasis on the reason for transplantation and vital signs (blood pressure, heart rate, respiratory rate) throughout the ICU stay.

2.2.2. Laboratory investigations

2.2.2.1. Routine Labs: CBC (complete blood count). Hemoglobin, Hematocrit, White blood cells, and platelet count; *Coagulation profile:* PT, PC, INR, and PTT; *Kidney Function Tests:* Na, K, creatinine, and Urea; and *Liver Function Tests*: ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), alkaline phosphatase, GGT (transaminase) total and direct bilirubin, total protein, and serum albumin. GFR was estimated with CKD-EPI equation expressed as a single equation: $\{GFR = 141 \times \min\ (S_{cr}/\kappa,\ 1)^{\alpha} \times \max(S_{cr}/\kappa,\ 1)^{-1.209} \times 0.993^{Age} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]\}$ where: S_{cr} is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1. All these measurements were made in a blind fashion by the hospital central laboratory as part of routine preoperative care [9].

2.2.2.2. Labs specific for this study. Urine samples for the determination of urinary NGAL and urinary creatinine were collected at four different time points: immediately before induction of anesthesia (the baseline NGAL), and 3, 18, and 24 h after reperfusion of the graft. Urinary creatinine level were determined by calorimetric method. Urinary NGAL/creatinine ratio was calculated to compensate for possible urinary dilution or concentration [7].

2.2.2.3. Urinary NGAL were measured by ELISA: [10]. The urine was collected aseptically directly into a sterile container, then centrifuged at 1000g for 10 min and the supernatant liquid was aliquot and stored at $\leqslant -20$ °C.

2.2.2.4. Reagent preparation. All reagents were brought to room temperature before use.

2.2.2.5. Wash Buffer. If crystals had formed in the concentrate, it was warmed to room temperature and mixed gently until the crystals had completely dissolved. A 20 mL of Wash Buffer Concentrate was added to deionized or distilled water to prepare 500 mL of Wash Buffer.

2.2.2.6. Calibrator Diluents RD5-24 (diluted 1:5). Diluents RD5-24Concentrate was added to 80 mL of demonized or distilled water to prepare 100 mL of Calibrator Diluents RD5-24 (diluted 1:5).

2.2.2.7. Substrate solution. Color Reagents A and B were mixed together. In equal volumes within 15 min of use, and protected from light. A 200 μL of the resultant mixture was required per well.

2.2.2.8. Lipocalin-2 standard. The Lipocalin-2 Standard was reconstituted with 1.0 mL of deionized or distilled water. This reconstitution was produced a stock solution of 100 ng/ml. The standard was mixed to ensure complete reconstitution and allowed to sit for a minimum of 15 min with gentle agitation prior to making dilutions. Pipette 900 μL of Calibrator Diluents' RD5-24 (diluted 1:5) into the 10 ng/mL tube. Pipette 500 μL of Calibrator Diluent RD5-24 (diluted 1:5) into the remaining tubes. The stock solution used to produce a dilution series. Each tube mixed thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (diluted1:5) serves as the zero standard (0 ng/mL) (see Fig. 1).

2.2.2.9. Assay procedure. The Lipocalin-2 Conjugate was remained at 2–8 °C during use, while all other reagents and samples must brought to room temperature before use. It was recommended that all samples and standards were assayed in duplicate. (Note: High concentrations of Lipocalin-2 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination). Excess micro plate strips were removed from

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