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Neutrophil gelatinase associated lipocalin as an indicator of acute kidney injury and inflammation in burned children

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ABSTRACT

Introduction: Neutrophil gelatinase associated lipocalin (NGAL) is a novel predictor of acute kidney injury (AKI), which increases with inflammation. We aimed to assess whether serum NGAL (SNGAL) and urine NGAL (UNGAL) can predict AKI in burned children.
Methods: Patients were referred within the 12 h of burn to our center. Serum samples for SNGAL, C-reactive protein (CRP), procalcitonin (PCT) and urine for UNGAL, microalbumine (Umalb), creatinine (Ucr) were obtained at both admission and the 5th day after burn. Blood urea nitrogen (BUN) and serum creatinine (Scr) were examined daily.
Results: Twenty-two subjects were enrolled and six (27.2%) of them developed AKI within the 48 h of injury. Burn size and abbreviated burn severity index (ABSI) were significantly increased in patients with AKI. CRP, PCT, SNGAL and UNGAL levels at admission and day 5 were significantly higher in patients with AKI than in those without AKI and controls. Scr was not significant between AKI and non-AKI groups at hospital days 1 and 5. A SNGAL level of 315 ng/ml and a UNGAL level of 100 ng/ml were determined as predictive cut-off values of AKI at admission (sensitivity and specificity: 71.4%, 83.3% and 93.3%, 93.7%, respectively). SNGAL and UNGAL were positively correlated with CRP, PCT, ABSI and Umalb/Ucr.
Conclusion: SNGAL and UNGAL are good early predictors of AKI in children with severe burn. NGAL might reflect the severity of burn insult and also could be used as an indicator of inflammation in burn children.

1. Introduction

Burn is not a simple injury that merely limited to skin. In moderate and severe burns systemic capillary leak occurs and multiple organ systems are usually involved as a result of endothelial dysfunction, inflammatory and hypermetabolic responses [1,2]. Kidneys are frequently affected [3]. Two forms of burn related acute kidney injury (AKI) have been described depending on the time of onset. Early-onset of AKI is mainly caused by intravascular hypovolemia, systemic vasoconstriction and myoglobinuria and appears during the first 5 postburn days. Late-onset of AKI is multifactorial, usually associated with sepsis and nephrotoxic drugs and occurs after 5 postburn days [4,5].

The current diagnosis of AKI depends on the measurement of serum creatinine (Scr). However, Scr is a delayed and unreliable indicator of AKI. Several factors such as age, gender,

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muscle mass and tubular secretion influence Scr. In addition, Scr may not change until a significant amount of renal function has been lost. Scr concentrations may not reflect the accurate decrease in glomerular filtration rate (GFR) during the acute changes in kidney function until a steady state equilibrium has been reached [6–8].

Neutrophil gelatinase associated lipocalin (NGAL) found in granules of neutrophils, is normally expressed at very low levels in various tissues and markedly induced by epithelial injury [7,8]. Several studies indicated that NGAL was an early diagnostic biomarker for AKI in common clinical AKI scenarios including contrast nephropathy, cardiac surgery, critical care and transplantation [9–12]. NGAL released by activated neutrophils has also been considered as a marker of bacterial infection and systemic inflammation in studies [13–15]. However, the data of NGAL in burn injury is limited [16]. Based upon the evidence of kidney dysfunction and inflammation in burn injury, we aimed to assess serum and urine NGAL levels of burn children with and without AKI.

2. Materials and methods

2.1. Patients and study design

This prospective study was conducted in the Burn Intensive Care Unit (ICU) in Cukurova University Hospital between 2009 and 2011. Age and gender matched 22 burn and 21 healthy children were included in the study. Patients were enrolled within the 12 h of burn injury. Total burn surface area (TBSA) and abbreviated burn severity index scores (ABSI) were recorded [17]. Exclusion criteria were superficial burns, TBSA below 10%, concomitant trauma, and preburn systemic or renal disease.

Patients were treated according to burn ICU protocols. Initial fluid resuscitation was performed with Ringer Lactate-5% Dextrose based on the Parkland Formula (4 ml/kg/TBSA%) and modified by according to hemodynamic and clinical status of each children. Enteral nutrition was immediately started and no prophylactic antibiotics were used. Surgical procedures (debridement, grafting) and regular dressing were applied.

The presence of AKI was identified by using the RIFLE (Risk; Injury; Failure; Loss; End-stage renal disease) classification system based on the changes in Scr and urine output. RIFLE risk was defined by 50% increase in baseline Scr and oliguria (urine output < 0.5 ml/kg/h for 6 h), RIFLE injury by 2 folds increase in baseline Scr and oliguria (urine output < 0.5 ml/kg/h for 12 h) and RIFLE failure by 3 folds increase in Scr and oliguria (urine output < 0.5 ml/kg/h for 24 h) or anuria for 12 h. When the data of urine output was unavailable, RIFLE grading was only made with Scr [18]. The preinjury baseline Scr values were not available and baseline Scr was estimated by using the lowest measured Scr in the first week of hospitalization as previously described [19]. Children who met any of the criteria for the RIFLE classification were defined as AKI patients.

The project was approved by the ethics committee of Cukurova University Hospital and written informed consent was received from at least one of the parents in all cases.

2.2. Assays

Blood and urine samples were obtained for the determination of NGAL level on admission (day 1) and fifth hospital day (day 5). Blood samples were immediately centrifuged at 3500 rpm for 5 min. After serum samples were achieved, they were frozen in 2-ml tubes and stored at $-80\,^{\circ}C$ until the test day. Complete blood count, blood urea nitrogen (BUN) and Scr were daily measured at 8.00 AM. C-reactive protein (CRP), procalcitonin (PCT), urine microalbumine (Umalb) and urine creatinine (Ucr) were analyzed on admission and day 5. The same parameters were measured in healthy children. Estimated glomerular filtration rate (eGFR) was calculated according to new Schwartz formula in both patient and control group [eGFR (ml/min/1.73 m^2) = 0.413 $\times$ height (cm)/Scr (mg/dl)] [20].

White blood cell (WBC) count was evaluated with a Beckman Coulter Hematology Analyzer. Serum CRP levels (in mg/l) were determined by a nepholometric method on a Beckman Coulter AU analyzer (Beckman Coulter, Ireland). A value of 5 mg/l was accepted as normal. Serum PCT levels were measured by a turbidimetric assay (B.R.A.H.M.S. PCT Sensitive Kryptor, B.R.A.H.M.S. AG, Henningsdorf, Germany). A level below than 0.5 ng/ml was considered as normal. Microalbuminuria (MA) was defined as Umalb to Ucr ratio $\geq$30 mg/g creatinine [21].

Urine and serum samples were kept in room temperature prior to NGAL analyses. Urine and serum NGAL levels were measured with Human Lipocalin-2/NGAL ELISA kit (BioVendor Labarotory Medicina, Chech Republic) and a solid-phase, two-side chemiluminescent enzyme immunometric assay (Immulite 1000 automated analyzer; Siemens Medical Solutions Diagnostics, Los Angeles, CA). The lower limit was 0.02 ng/ml for both serum (SNGAL) and urine NGAL (UNGAL). To standardize the samples, UNGAL levels were expressed as ratio of NGAL to urine creatinine (UNGAL/Ucr, ng/g).

2.3. Statistical analysis

Statistical analysis was done by using SPSS version 18.0 for Windows. Assumptions of normality and homogeneity of variance were initially checked. The differences between two groups were assessed with using t test or Mann–Whitney U test, as appropriate. One way ANOVA and/or Kruskal Wallis test was used to compare the parameters between three groups. Data were presented as median and ranges. Categorical variables were expressed as proportions and compared with using Chi-square test. Correlations among variables were assessed using the Spearman rank coefficient. A receiver operating characteristic (ROC) curve was constructed to determine the cut-off value of SNGAL and UNGAL as a biomarker of AKI. Their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio (LR) and 95% confidence interval were calculated. A value of $p < 0.05$ was considered statistically significant for all tests.

3. Results

The current study involved 22 subjects (14 boys, 8 girls) and 21 healthy children (12 boys, 9 girls). Six (27.2%) developed