COMPARISON OF URINE WITH PLASMA NEUTROPHIL GELATINASE–ASSOCIATED LIPOCALIN IN DETECTING ACUTE KIDNEY INJURY AFTER CARDIOPULMONARY BYPASS SURGERY

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ABSTRACT

Objective: To compare the accuracy of urine with plasma neutrophil gelatinase-associated lipocalin (NGAL) in early detection of acute kidney injury (AKI) following cardiopulmonary bypass (CPB) surgery.

***Study Design*:** A prospective cohort study.

Place and duration of study: Department of Chemical Pathology and Endocrinology, AFIP from December 2011 to July 2012.

Patients and Methods: Ninety three adult patients planned for CPB surgery in AFIC/NIHD were consecutively included. Blood for serum creatinine were collected preoperatively, 4, 24 & 48 hours (h) after CPB surgery. Blood and urine samples for NGAL analysis were collected only at 4 h. Serum creatinine, plasma and urine NGAL samples were analyzed on UniCel® DxC 600 (Beckman), TRIAGE meter pro (Biosite) and ARCHITECT i2000SR analyzer (Abbott) respectively.

Results: Out of 93 patients undergoing CPB surgery, 12 (13%) developed AKI. AKI patients had significantly higher median interquartile range (IQR) urine NGAL of 180 ng/ml (105-277 ng/ml) as compared to control of 6 ng/ml (2-15 ng/ml) and median plasma NGAL of 170 ng/ml (126-274 ng/ml) as compared to control of 75 ng/ml (61-131 ng/ml). The patients had increased urine vs plasma NGAL area under curve (AUC) [0.91 vs 0.70 (p = <0.001)], better sensitivity (91% vs 82%) and specificity (98% vs 65%).

Conclusion: Plasma and urine NGAL values increased significantly in AKI patients as compared to serum creatinine values. Urine in comparison to plasma NGAL revealed more sensitivity and specificity in detecting AKI following CPB surgery.

Keywords: Neutrophil gelatinase–associated lipocalin, cardiopulmonary bypass surgery, acute kidney injury.

INTRODUCTION

Acute kidney injury (AKI) is an overwhelming problem of critical care setting leading to increased morbidity and mortality if not addressed on time. A common complication of post cardiopulmonary bypass (CPB) surgery in adults is AKI, affecting 40% of patients and requiring dialysis in 1% of cases[1]. Rapid detection of kidney injury has been long required and neutrophil gelatinase–associated lipocalin (NGAL) has been shown to be the biomarker that could fill this diagnostic gap as compared to serum creatinine which showed a delayed rise in the early hours of kidney dysfunction[2].

Urine NGAL has been widely studied for early detection of AKI, a 100 ng/ml cutoff value at 2 hours showed an area under curve (AUC) of 0.95 with a sensitivity of 82% and specificity of 90%[3]. Plasma NGAL is also considered as a powerful independent predictor of AKI after CPB surgery. For prediction of AKI, plasma NGAL cutoff value of 150 ng/ml at 2 hours showed AUC of 0.96 with a sensitivity of 84% and specificity of 94%[4].

Most studies use cumbersome enzyme-linked immunosorbent assay technique that is difficult to implement in routine practice[5]. However commercial kits for auto analyzers have been developed for determination of urine NGAL using chemiluminescent microparticle immunoassay (CMIA) technology. Similarly, commercial kits for point of care testing device have also been developed for quantitative detection of plasma NGAL, which utilizes fluorescence immunoassay.

The objective of this study is to compare the diagnostic ability of urine with plasma

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NGAL for establishing AKI following cardiac bypass surgery for use especially in intensive care settings. Our study will facilitate clinicians and intensivists in managing AKI cases in time and effectively for a better outcome post CPB before irreversible kidney injury occurs.

PATIENTS AND METHODS

We conducted this prospective research work at the department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi in collaboration with the Armed Forces Institute of Cardiology (AFIC) / National Institute of Heart Disease (NIHD), Rawalpindi after approval of the institutional review committee. Ninety three patients (18-80years) admitted for elective CPB surgery in AFIC/NIHD were consecutively enrolled. Informed consent was taken from the patients. Patients using nephrotoxic drugs, suffering from chronic kidney disease, peripheral vascular disease and those who had kidney transplant were excluded.

Samples for serum creatinine were drawn in plain tubes preoperatively, 4, 24 & 48 hours after CPB surgery whereas blood samples for plasma NGAL were drawn at 4 hours in ethylenediamine tetraacidic acid (EDTA) tube. Serum and plasma were separated, stored at – 80 °C until assayed. Urine samples were collected 4 hours after bypass surgery in plain tubes, immediately centrifuged for 5 minutes at 5,000 g and clarified specimens were transferred to secondary tubes for storage at – 80 °C until assayed.

Samples of all the patients were analyzed at AFIP. Plasma samples were analyzed on TRIAGE meter pro (Biosite Inc., San Diego, California, USA). Urine NGAL was measured via ARCHITECT assay on ARCHITECT I 2000 SR analyzer (Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IIIinois, USA) using chemiluminescent microparticle immunoassay (CMIA) technology. Creatinine samples were measured via Synchron assay on UniCel® DxC 600 Synchron® Clinical System (Beckman Coulter Inc., Fullerton, California, USA).

Acute kidney injury network criteria (AKIN) were used for establishing AKI: an increase in serum creatinine levels of more than 0.3 mg/dl (26.3 µmol/l) , more than 50% rise from baseline serum creatinine, or oliguria of < 0.5 ml/kg/h for more than six hours[6].

Statistical package for social sciences version 19.0 (SPSS Inc, Chicago, IL, USA) was used for analysis of the data. The result was expressed in median [inter quartile ranges (IQRs 25-75%)] for quantitative variables. The Spearman rank order correlation was used to assess the correlation between urine and plasma NGAL. Plasma and urine NGAL were compared for detecting AKI using receiver operating characteristic (ROC) curve with optimal cutoffs, sensitivity and specificity. *p*-value <0.05 was considered statistically significant.

RESULTS

Ninety three patients were studied and grouped into AKI (n=12) and non AKI (no AKI=81) depending on AKIN criteria, after fulfilling inclusion and exclusion criteria. There was statistically significant (p = 0.000) absolute (in µmol/l) and relative (%) rise in serum creatinine in AKI vs non AKI group from baseline serum creatinine levels following cardiac bypass(Table-1). Out of 12 patients, four patients had a 50 % increase in serum creatinine value as well as 26.3 µmol/L rise from baseline. Whereas remaining eight had only 26.3 µmol/L increase from baseline to peak levels.

Median (IQR) urine NGAL level of 180 ng/ml (105-277 ng/ml) in AKI group was significantly higher (p= 0.000) than 6 ng/ml (2-15 ng/ml) in non AKI group. Similarly, AKI patients showed significantly raised median (IQR) plasma NGAL of 170 ng/ml (126-274 ng/ml) vs 75 ng/ml (61-131 ng/ml) in non AKI group (fig. 1). Spearman rank order correlation analysis was utilized to see plasma and urine NGAL ability to predict severity of clinical outcome. There was a positive correlation between urine and plasma NGAL which was statistically significant (rs = 0.991, p = 0.000).

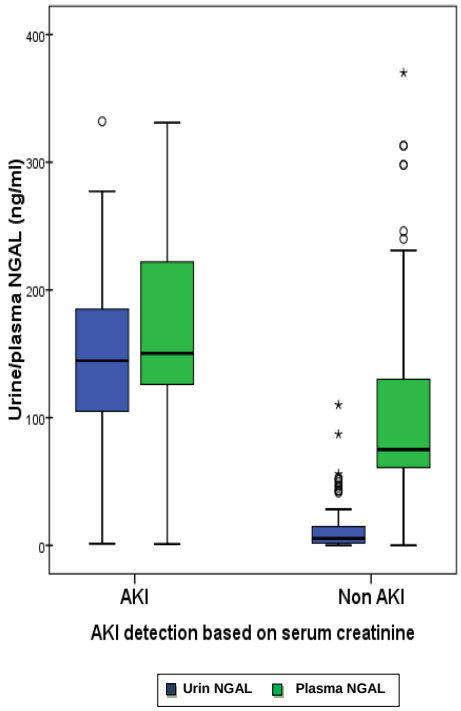
Urine and plasma NGAL ability to predict AKI at various cutoff points was assessed using

ROC curves. At a cutoff value of 87 ng/ml urine NGAL showed sensitivity of 91% [95% confidence interval (CI) 59-98], specificity of 98% (95% CI 92-99), positive likelihood ratio (LR) of 70% (95% CI 58-85), negative LR of 0.092% (95% CI 0.006-1.4), area under the receiver operator characteristic curve (AUC) of 0.91 (95% CI 0.84-0.96) with standard error (SE) of 0.06 at 4 h after CPB surgery. For plasma NGAL a cutoff of 123 ng/ml yielded sensitivity of 82% (95% CI 48-97), specificity of 65% (95% CI 53-76), positive LR of 2% (95% CI 2-3.2), negative LR of 0.28% (95% CI 0.08-1.0), AUC of 0.70 (95% CI 0.58-0.78) and SE of 0.093 at 4 hours after CPB surgery. Comparison of urine and plasma NGAL ROC curves show the difference between areas of 0.22, standard error of 0.05 and the significance level of < 0.001 (fig. 2).

Table-1: Characteristics of AKI and non AKI patients in cardiopulmonary bypass surgery patients.

Parameter	No AKI (n = 81) Median (IQR)	AKI (n = 12) Median (IQR)	*p*-value
Age(y)	50 (45-61)	56 (46-64)	NS
Preoperative eGFR (ml/min/1.73 m²)	82 (66-94)	70 (56-85)	NS
Baseline serum creatinine (umol/l)	89 (75-98)	97 (81-114)	NS
Serum creatinine 48 hours (umol/l)	91 (79-106)	134 (123-164)	<0.000
Change in serum creatinine Relative (%) Absolute (umol/l)	6 (0-22) 6 (0-15)	45(32-56) 44 (32-52)	<0.000 <0.000

Figure-1: Urine NGAL and plasma NGAL values were use to detect AKI vs no AKI based on serum creatinine.



DISCUSSION

Acute kidney injury is a complex disorder with no uniform definition. Current dilemma is lack of availability, consensus and standardization of suitable real-time markers for establishing AKI. It contributes to morbidity and mortality significantly. Creatine which is a clearance marker rather than an injury marker is used for establishing AKI. It is for this reason

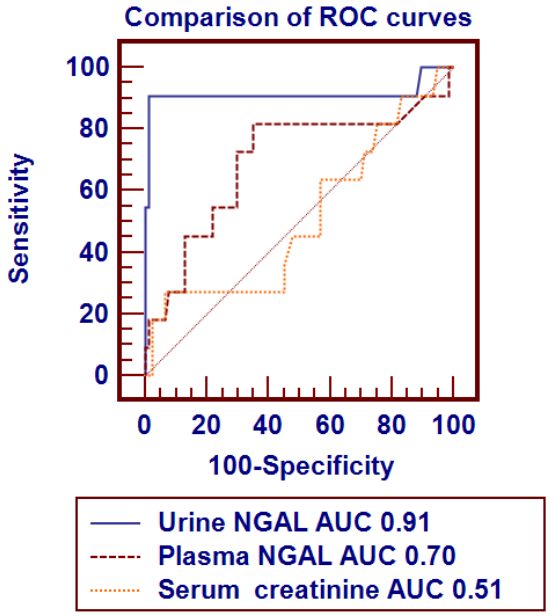


Figure-2: Comparison of receiver operating characteristic (ROC) curves of urine NGAL, plasma NGAL and serum creatinine levels at 4 hours after CPB. NGAL, neutrophil gelatinase-associated lipocalin; CPB, cardiopulmonary bypass.

that many urinary and plasma biomarkers have been introduced to address this issue. Interleukin-18, urine/plasma NGAL and cystatin C are among those[7-8]. Previously used urinary biomarkers like fractional excretion of Na and cats have poor sensitivity and specificity. Distal neurons produce NGAL lipocalin which has gained great attention in the current decade because of its expected ability for early detection of AKI. It is a rapidly induced protein resulting in its appearance in urine soon after a kidney injury. Its role in AKI was investigated in various settings like in cardiac surgery, inflammation, sepsis and intensive care patients.

These biomarkers need to be validated for use in clinical settings. They appear in urine and plasma at least 24 hours before the serum creatinine shows a rise[9]. There was greater than 10 fold increase in plasma NGAL and more than 100 fold increase in urine NGAL in patients of AKI after ischemia or sepsis as compare to controls[10]. In this research work, we compared both NGAL markers for a significant rise in AKI group. Utilizing NGAL for detecting AKI in ICU setting de Geus et al. Studied 632 patients, of whom 171 (27%) developed AKI. In that study urine and plasma NGAL proved to be of great utility in predicting AKI for RIFLE injury class with an AUC of 0.85 and 0.80 respectively[11].

Raised levels of urine and plasma NGAL post CPB has been observed in various studies in patients who subsequently developed AKI[12-14]. However it was observed that AUC-ROC in studies conducted for predicting AKI on adult population has a wider range and values were lower as compared to those in pediatric group[15-16]. Diagnostic and prognostic utility of NGAL has been addressed in a systematic review and meta-analysis. Urine (AUC 0.83) and plasma NGAL (AUC 0.77) diagnostic accuracy was found to be similar as is the case in our research findings[17].

Our study has several strengths. Patients were not suffering from AKI preoperatively. Plasma NGAL can be used on patient bedside as a point of care testing device, a patient suffering from oliguria can also be monitored by utilizing plasma and Triage device can be utilized in smaller setups. For urine, it is easy to collect and more fold rise is seen in urine NGAL as compared to plasma NGAL depicting more sensitivity. There are certain limitations of our study. Most of the patients have co-morbid conditions like diabetes mellitus, old age, hemodilution and emergency surgery. Creatinine which itself is a poor marker was used as gold standard.

NGAL is the most promising of all new biomarkers. However its maximum utility can be availed when used in combination with other biomarkers that is 'AKI panel'[18-19]. Comparing urine with plasma NGAL has helped in more robust utilization of these biomarkers in clinical settings, especially in cardiac surgery. However our study needs to be validated in different settings along with other biomarkers of AKI for better patient outcome.

CONCLUSION

Plasma and urine NGAL values increased significantly in AKI patients as compared to serum creatinine values. Urine in comparison to plasma NGAL revealed more sensitivity and specificity in detecting AKI following CPB surgery.

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