ORIGINAL ARTICLE

Comparison of Neutrophil Gelatinase Associated Lipocalin with Serum Creatinine for Early Detection of Decrease in Estimated Glomerular Filtration Rate

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ABSTRACT:

Objective: To determine the accuracy of Neutrophil Gelatinase Associated Lipocalin (NGAL) at 2 h compared to serum creatinine at 48 h after Computerized Tomography (CT) scan with contrast medium for early detection of decrease in estimated Glomerular Filtration Rate (eGFR).

Materials and Methods: This Cross Sectional Study was carried out in Departments of Chemical Pathology and Radiology PNS SHIFA Karachi from 1st February 2014 to 31st Jan 2015. One hundred and ten patients undergoing CT scan with contrastwere included. Serum creatinine wasmeasured byJaffé assay using Alkaline Picrate reagent on Modular P800 (Roche) analyzer before and 48 h after CT scan. eGFR was calculated by Modification of Diet in Renal Disease (MDRD) formula. Patients were divided into AKI and non-AKI groups. NGAL was analyzed by NGAL Reagent Kit on Triage meter pro at 2 h after contrast.

Results: Out of 110 patients, 5 (4.54%) developed Acute Kidney Injury (AKI) 2 h after CT scan with contrast in which eGFR was decreased more than 25%. NGAL level in AKI group was 161ng/ml (IQR: 98 to 196) at 2 h after CT contrast was significantly higher than non-AKI group of 63ng/ml (IQR: 42 to 75). Serum creatinine showed significant rise but with delay of 24 – 48h after CT contrast in AKI group. There was a positive correlation between 2 h plasma NGAL and eGFR at 48 h, which was statistically significant.

Conclusion: NGAL is a potential marker for early detection of decrease in eGFR and predictor of AKI.

Keywords: Acute kidney injury, Biomarkers, Creatinine, Estimated Glomerular Filtration Rate, Neutrophil Gelatinase Associated Lipocalin, CT scan with contrast.

INTRODUCTION:

Acute Kidney Injury (AKI) is a variety of severity of stages of kidney dysfunction ranging from a reversible decline in the glomerular filtration rate (GFR) to sustained Acute Renal Failure (ARF) with anuria, which may progress to chronic renal failure[1]. In 2004 the Acute Dialysis Quality Initiative Group developed a new definition for ARF, called the Risk (R), Injury (I), Failure (F), Loss of kidney function (L) and End-stage kidney disease (E) (RIFLE) criteria[2]. Later, for the term ARF, Acute Kidney Injury Network (AKIN) criteria (AKI) was used, reflecting the fact that structural injury most certainly precedes an acute decline in kidney function. The criteria is identical to the first three stages of RIFLE, with the exception of a shorter time frame of AKI within 48 hours, and a lower creatinine threshold of greater

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than 26.4 umol/L from baseline to peak value[3]. Serum creatinine levels and changes in urine output have been used to define AKI for decades. Typically, the serum creatinine level begins to increase 24-72 hours after AKI, peaks at 3-5 days and requires further 3-5 days to return to baseline[4]. Creatinine is now considered as an inadequate marker, as serum creatinine levels rise when more than 50% of kidney functions are lost. Moreover serum creatinine is not specific for AKI[5]. Neutrophil Gelatinase Lipocalin (NGAL), was first purified from the secondary granules of human neutrophils[6]. NGAL is among the most promising biomarker after ischemic AKI in animal models and further experimental studies[7]. As the contrast induced AKI can easily be studied in hospital settings, so we compared accuracy of NGAL and serum creatinine to detect AKI after CT scan with contrast. This study was undertaken to determine the accuracy of Neutrophil Gelatinase Associated Lipocalin (NGAL) at 2 h compared to serum creatinine at 48 h after CT scan with contrast for early detection of decrease in estimated Glomerular Filtration Rate (eGFR).

MATERIALS AND METHODS:

This Cross sectional study was carried out in Department of Chemical Pathology, PNS SHIFA Karachi in collaboration with Department of Radiology, from 1stFebruary 2014 to 31stJan 2015. According to the RIFLE criteria, AKI is an abrupt reduction in kidney function, evidenced by decrease in eGFR of more than 25%[3]. ForNGAL, a cutoff value of 100 ng/ml in serum after 2 h of iodinated contrast medium was taken as AKI[4].

110 subjects of either sex scheduled for contrast enhanced CT of any part of the body, with contrast dose (Iobitridoil) of more than 90ml, ages between 18 to 80 years were selected by non-probability consecutive sampling. Individuals with advanced chronic kidney disease i.e. serum creatinine greater than 300 ìmol/L or patients on chronic hemodialysis, with kidney transplant, with peripheral vascular disease and on nephrotoxic drugs were excluded from the study. The study was conducted after approval by the Ethical Committee of PNS-SHIFA. All subjects fulfilling the inclusion criteria were elaborately apprised about the study to obtain their informed consent. The name, age, gender and contact numbers were noted. Medical history and physical examination was carried out and blood samples were collected. Blood samples of subjects for NGAL and creatinine were drawn from ante cubital vein aseptically before CT scan with contrast, 2 h and 48 h after CT scan. NGAL samples collected in EDTA tubes while serum creatinine samples were collected in plain serum tubes.

NGAL was analyzed by means of NGAL Reagent Kit on Triage meter pro which utilizes a two-step immunoassay for using chemiluminescent micro particle immunoassay (CMIA) technology[5]. Serum creatinine was measured by the Jaffé assay using Alkaline Picrate on Modular P800 fully automated chemistry analyzer (Roche) using Roche calibrator and controls as per manufacturer instructions and specifications[6]. eGFR was calculated by Modification of Diet in Renal Disease (MDRD) formula[7]. Patients were divided into AKI and non-AKI groups on the basis of 25% reduction in eGFR[8]. All the data was entered in a specially designed proforma and analyzed using SPSS version 18. Descriptive statistics for qualitative variables were calculated in percentage. Mean and SD were calculated for all quantitative variables like age, NGAL levels and serum creatinine levels at 2 h and 48 h.

RESULTS:

A total of 110 patients undergoing CT scan with contrast who met the inclusion and exclusion criteria were consecutively included. There were 68 males (61.8%) and 42 females (38.2%). The age of the patients ranged from 18 to 71 years, with mean age of 52 years. Subjects were grouped into AKI and Non AKI group as per patient characteristics of the study based on RIFLE criteria and AKIN criteria. Serum creatinine levels prior to CT contrast and at 2 h after the contrast, did not show any significant difference between two groups (Table 1).Whereas serum creatinine levels at 48 h and plasma NGAL levels at 2 h after the CT contrast showed significant statistical difference between two groups (Table I).

Table: 1

Characteristics of CIN after CT without AKI as compared with the patients who developed AKI within 48 h

Parameter	Non AKI (n = 105) Median (IQR)	AKI (n = 5) Median(IQR)	P value
Gender (number)	Male (65), Female (40)	Male (03), Female (02)	-
Age (years)	48 (36 to 59)	59 (50 to 65)	-
Baseline eGFR ml\min\1.73m^2	81 (90-71)	71 (76-62)	-
eGFR at 48 h ml\min\1.73m^2	77 (83-61)	49 (51-38)	-
Baseline serum creatinine (umol/L) before CT contrast	92 (84 to 103)	99 (94 to 112)	< 0.270
Serum creatinine (umol/L) at 2 h after CT contrast	94 (84 to 103)	102 (94 to 112)	<0.310
NGAL at 2h (ng/ml) after CT contrast	63 (42 to 75)	161 (98 to 196)	<0.0001
Serum creatinine at 48 h (umol/L) after CT contrast	96 (105-117)	137 (132 to 164)	<0.0001

Five patients (4.53%) developed AKI fulfilling the RIFLE criteria and their eGFR decreased by more than 25% as shown in Table 1. Plasma NGAL ability was assessed to predict AKI after CT with contrast. NGAL levels in AKI group of 161ng/ml (IQR: 98 to 196) at 2 h after CT contrast were significantly higher as compared to non-AKI group having a level of 63ng/ml (IQR: 42 to 75), with a p value of < 0.0001. Serum creatinine

showed significant rise with a delay of 24 to 48 h in AKI group (Table 1).

Plasma NGAL ability to predict clinical outcome was assessed using Spearman rank order correlation analysis. There was a positive correlation (Figure 1) between 2 h plasma NGAL and serum creatinine at 48 h after CT contrast, which was statistically significant (rs = 0.33, P = < 0.001). The Independent sample Mann Whitney U Test also showed that plasma NGAL at 2 h and serum creatinine at 48 h after the CT contrast reject the null hypothesis while creatinine at 2 h after CT contrast retains the null hypothesis (Table 2).

Figure: 1

Graph showing comparison between NGAL after 2 h and creatinine at 48h after CT contrast.

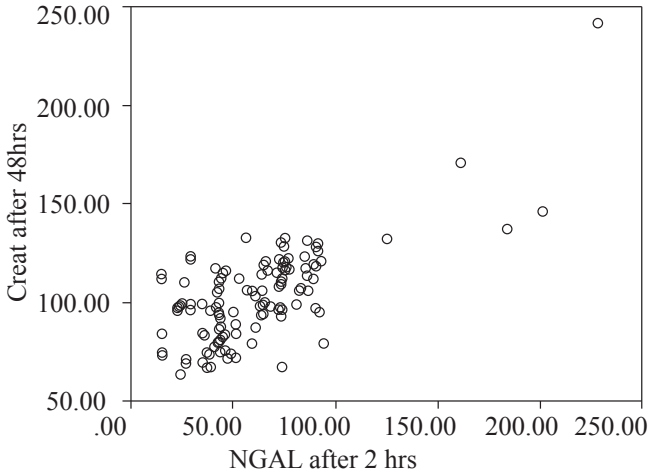

Table: 2

Comparison of NGAL and creatinine at 2 h after CT contrast and creatinine at 48 h after CT contrast by independent sample Mann Whitney U Test

Null Hypothesis	Test	Sig	Decision
The distribution of creatinine at 2 h is same across categories of AKI criteria	Independent sample Mann Whitney U Test	0.119	Retain the null
The distribution of NGAL after 2 h is same across categories of AKI criteria	Independent sample Mann Whitney U Test	0.000	Reject the null hypothesis
The distribution of Creatinine after 48 h is same across categories of AKI criteria	Independent sample Mann Whitney U Test	0.000	Reject the null hypothesis

DISCUSSION:

AKI is characterized by abrupt and sustained decline in GFR. The absence of a uniform definition has, however, delayed the ability to compare preventive strategies, therapies and outcomes in different studies[9,10]. According to the RIFLE criteria AKI is an abrupt reduction in kidney function, evidenced by decreased in GFR of more than 25% within 48 h after the administration of contrast agent. Serial serum creatinine measurements are taken as gold standard for identification and classification of AKI but this may be problematic for a number of reasons such as serum creatininelevels rise when more than 50% of kidney functions are lost, serum creatinine is not specific for kidney injury, serum creatinine level begins to increase 24-72 hours after AKI, it peaks at 3-5 days and requires further 3-5 days to return to baseline etc.[11]. Levels can vary widely depending on non-renal factors such as gender, muscle mass and hydration status. Conventional urine markers such as casts or fractional excretion of sodium are insensitive and non-specific for early recognition of AKI[12]. As in RIFLE criteria AKI is defined on the basis of creatinine which is a poor marker of AKI, so it causes late diagnosis and bad prognosis of AKI. There are several new biochemical markers of tubular damage which include Cystatin C, IL -18, NAG, KIM -1, L- FABP, homocystiene, á1-microglobulin and NGAL[13]. NGAL, also known as human neutrophil lipocalin or lipocalin [2], was first purified from the secondary granules of human neutrophils in the search for ideal biomarkers of AKI. NGAL was among the most promising biomarker of AKI. In our study Plasma NGAL ability to predict clinical outcome was assessed using Spearman rank order correlation analysis. There was a positive correlation between 2 h after CT contrast plasma level of NGAL and serum creatinine at 48 h after CT contrast, which was statistically significant (rs = 0.33, p = 0.001). Our study showed that at cut off level of 99ng/ml NGAL rejected the null hypothesis by Independent Mann Whitney U Test while creatinine at 2 h after CT contrast retained the null hypothesis. These were the properties of early and excellent biomarker in detecting AKI. Documented studies of establishment of NGAL as marker of AKI after contrast medium are scarce to nil in Pakistan. A study was carried out by Usman et al to determine the accuracy of NGAL in early detection of AKI after cardiopulmonary bypass surgery by comparing with serum creatinine[14]. Analysis of urine NGAL at a cutoff value of 87 ng/ml showed area under the curve of 0.91 [95% confidence interval (CI) 0.83 – 0.96] with sensitivity of 90.9% (95% CI 58.7 – 98.5) and specificity of 98.7% (95% CI 92.9-99.8).

The first study evaluating NGAL as an AKI predictor was conducted on children after cardiac surgery. Urinary NGAL rose almost 100-fold and serum NGAL 20-fold up to 48 h (ROC of 0.998) before AKI was detected by creatinine[15]. Siew et al. reported a (ROC) AUC ¼ 0.77 (CI 0.64–0.90) for developing AKI in a subgroup of patients for urine NGAL[16]. The predictive performance was also highlighted in a meta-analysis pooling data from 19 studies and eight countries involving 2,538 patients[17]. The overall AUROC for AKI prediction was 0.815 and was similar in general ICU patients. Several studies such as Liangos et.al, showed that in 103 cardio pulmonary bypass patients 2 h after surgery, AUC for NGAL was ¼ 0.50 with CI 0.33–0.68[18]. Koyner et al. measured both plasma NGAL AUC 0.526 (0.388–0.664) and urinary NGAL AUC ¼ 0.705 (CI 0.581–0.829) at ICU admission[19]. Research articles published have

showed that both urinary NGAL and plasma NGAL are superior to other emerging biomarkers of AKI.[20,21,22,23] It has been documented that among biomarkers for AKI, NGAL is best available biomarker for AKI. These results are quite similar to results of our study.[24,25]

CONCLUSION:

NGAL seems to be a potential marker for early detection of decrease in eGFR and predictor of AKI. As a biomarker of AKI it is far superior in comparison to serum creatinine. It is particularly important in reducing the expenditure of stay in hospital by early diagnosis of AKI. It can fulfill the gap of early biomarker of AKI. Further studies should be done for establishment of role of NGAL in AKI in Pakistan especially in patients with pre-existing decreased eGFR.

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