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PLASMA NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN IN THE EARLY DETECTION OF ACUTE KIDNEY INJURY IN PATIENTS UNDERGOING CARDIAC SURGERY

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ABSTRACT

The aim of this study is to determine that plasma Neutrophil Gelatinase Associated Lipocalin (NGAL) detects AKI earlier in patients undergoing cardiac surgery compared to serum creatinine. It is a single centre, case control study, where 50 controls (apparently healthy individuals) and 53 cases (adults who underwent major cardiac surgeries) were chosen as study group. Plasma NGAL and serum creatinine were estimated in random samples for controls. NGAL was estimated preoperatively and at 4 hours post operatively for the study group. Creatinine was estimated preoperatively and at 4 hours, 24 hours, 48 hours and 72 hours post operatively for the study group. Plasma NGAL was measured using ELISA and creatinine using modified kinetic Jaffe method. Paired T test was used to analyse different variables. NGAL post operative values at 4 hours were higher than the NGAL pre operative values (p=0.00). Creatinine post operative values at 4 hours (p=0.28) and 24 hours (p=0.30) were not higher whereas at 48 hours (p=0.04) and 72 hours (p=0.01) were higher. Among the 53 patients, 13 were classified as AKI based on AKIN criteria. Creatinine levels were elevated at 48 hours and NGAL levels were elevated at 4 hours in patients with AKI. NGAL was increased at 4 hours and creatinine at 48 hours in patients with AKI, indicating NGAL can detect AKI earlier compared to creatinine.

KEYWORDS: AKI, Cardiac surgery, Creatinine, NGAL

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INTRODUCTION

Diseases of the kidney are diverse, ranging from Acute Kidney Injury (AKI) to End Stage Renal Disease (ESRD). AKI refers to a syndrome that results from multiple causative factors and occurs in a variety of clinical settings. AKI presents with varied clinical manifestations that range from a minimal elevation in serum creatinine to anuria [1]. An acute deterioration in renal function is a common complication after cardiac surgery, with an incidence of 7.7% to 42% depending on the criteria used to define AKI [2-6]. AKI is associated with a complicated clinical course such as prolonged intensive care and hospital stay, dialysis dependency and long term mortality [7]. AKI is largely asymptomatic and establishing the diagnosis relies on functional biomarkers such as serial serum creatinine measurements [1]. Unfortunately, serum creatinine is a delayed and unreliable indicator of AKI, does not accurately depict kidney function until a steady state has been reached which could take several days [8-11]. Fortunately, understanding the early stress response of the kidney to acute injury has revealed a number of potential biomarkers. Neutrophil Gelatinase Associated Lipocalin (NGAL) is one among them [1]. Human NGAL was originally identified as a novel protein isolated from secondary granules of human neutrophils [12]. It is a 25kDa protein covalently bound to neutrophil gelatinase [13]. NGAL has been extensively investigated as an early biomarker for AKI in various clinical settings like cardiac surgery, intensive care unit, contrast induced nephropathy, kidney transplantation etc. NGAL also satisfies a number of characteristics of an ideal AKI biomarker. These include non-invasiveness, rapidity of measurements, sensitive to facilitate early detection and allow for risk stratification, amendable to clinical assay platforms and the results predict clinical outcomes, efficacy of therapies and expedite drug development process [1]. This study highlights the use of plasma NGAL in the early prediction of AKI in patients undergoing cardiac surgery.

MATERIALS AND METHODS

It was a single centre case control study conducted at the Department of Cardiothoracic and Vascular Surgery, Sri Ramachandra Medical College and Research Institute, Chennai. Fifty apparently healthy individuals in the age group of 18 to 60 years of either sex were chosen as the controls. Fifty three patients undergoing major cardiac surgeries like coronary artery bypass grafting, cardiac valve repair or replacement, repair of congenital heart defects / large septal defects with or without cardiopulmonary bypass in the age group of 18-60 years of either sex were chosen as the study group. Patients with pre existing renal disease, malignancies and emergency surgeries were excluded from the study. The study was conducted after obtaining Institutional Ethics Committee clearance. Each participant was explained about the details of the study and informed consent obtained. Blood samples were drawn randomly from the controls for estimating serum creatinine and plasma NGAL values. In the study group, plasma NGAL was estimated preoperatively (to establish a baseline value) and at 4 hours post operatively. Serum creatinine was estimated preoperatively, 4 hours, 24 hours, 48 hours and 72 hours post operatively. The blood samples were collected in violet topped vacutainers containing EDTA for plasma NGAL and yellow topped gel vacutainers for serum creatinine estimation. The samples were processed, separated and stored in storage vials at -70^0 C until analysis. Plasma NGAL was measured using NGAL ELISA KIT (KIT 036) from the Bioporto Diagnostics, Gentofte, Denmark. (LOT No: NG1108RUO) in Biorad Evolis version 1.55.1.Serum creatinine was estimated by modified kinetic Jaffee method using automated analyzer Dade Behring RXL Dimension Max.

RESULTS AND DISCUSSION

Stata / SE version 11.0 was used for all the statistical analyses. Table I shows the demographic data. Out of the 53 cases, 32 underwent CABG and 21 underwent valve replacements. Twenty four patients had on pump and twenty five had off- pump surgeries.

Extreme observations were removed from the dataset using box plot analysis since it skewed the distribution. Table II and table III shows the descriptive statistics of NGAL and creatinine values respectively. Paired T test was used to analyse different variables. No statistical significance of NGAL and creatinine in control group and preoperative samples of the study group were found. Table IV shows the results of preoperative and postoperative comparison. The plasma NGAL post operative 4 hour values was significantly higher than the plasma NGAL preoperative values (p=0.00). The serum creatinine values at 4 hours (p=0.28) and at 24 hours (p=0.30) after the surgery were not higher than the preoperative values, but the values at 48 hours (p=0.04) and 72 hours (p=0.01) after the surgery were significantly higher than the preoperative values.

Table I
Demographic data

		PATIENT	CONTROL
GENDER	MALE	37	29
	FEMALE	16	21
SURGERY	CABG	32	Not Applicable
	VALVE REPLACEMENT	21	
ON/OFF PUMP TECHNIQUE	ON PUMP	28	
	OFF PUMP	25	
COMORBIDITIES	DIABETES	32	
	HYPERTENSION	27	

CABG – Coronary Artery Bypass Grafting

Table II
Descriptive statistics showing NGAL values

Variable – NGAL	Observed	Mean (ng/ml)	Std. Err.	Std. Dev.	[95% Conf. Interval]	
CONTROL GROUP	49	94.71	4.77	33.35	85.13	104.29
PATIENT GROUP Preoperative	50	115.40	5.69	40.21	103.97	129.33
PATIENT GROUP Postoperative 4 hrs	50	181.90	9.03	63.88	163.74	200.06

Table III
Descriptive statistics showing Creatinine values

Variable CREATININE	Observed	Mean (mg/dl)	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Control Group	50	0.90	0.03	0.18	0.85	0.95
Patient group Preop	53	1.04	0.03	0.19	0.99	1.09
Patient group Postop – 4 hrs	53	1.07	0.03	0.19	1.01	1.12
Patient group Postop – 24 hrs	53	1.06	0.04	0.30	0.92	1.08
Patient group Postop – 48 hrs	53	1.13	0.04	0.32	1.04	1.22
Patient group Postop – 72 hrs	53	0.96	0.03	0.24	0.89	1.02

Preop – preoperative; Postop – postoperative

Table IV
Comparison of NGAL and creatinine of study and control group

Variable1	Variable2	p value
NGAL control	NGAL preop	0.90
Creatinine control	Creatinine preop	0.72
NGAL Preop	NGAL postop 4	0.00
Creatinine Preop	Creatinine postop 4	0.28
Creatinine Preop	Creatinine postop 24	0.30
Creatinine Preop	Creatinine postop 48	0.04
Creatinine Preop	Creatinine postop 72	0.01
Creatinine Postop 24	Creatinine postop 48	0.00

Preop – preoperative; Postop – postoperative. Preoperative and postoperative comparison in study group

Among the 53 patients who underwent cardiac surgery, 13 were classified under the AKI category based on the AKIN definition and staging criteria (increase in serum creatinine of ≥ 0.3 mg/dl developing over 48 hours). Figure 1 show the time series change in creatinine levels before and 4, 24, 48 and 72 hours after surgery. The serum creatinine levels for the patients with AKI were found to be higher than those without AKI. Also, the levels were high during the 48 hour time period. The change in NGAL levels before and 4 hours after surgery are shown in Figure 2. The plasma NGAL levels for the patients with AKI were higher than those without AKI. Also, the levels are significantly high during the 4 hour time period for the AKI patients and no significant elevation for the patients without AKI. Also, the significant elevation occurred at 48 hours for creatinine whereas the elevation was predominant at 4 hours for NGAL values.

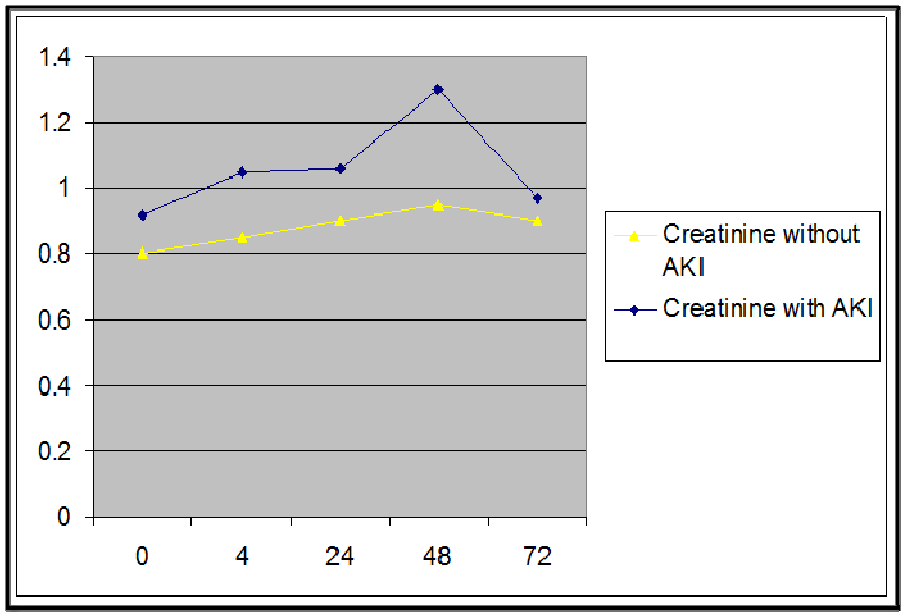

Figure 1
Creatinine at various time points (with & without AKI)

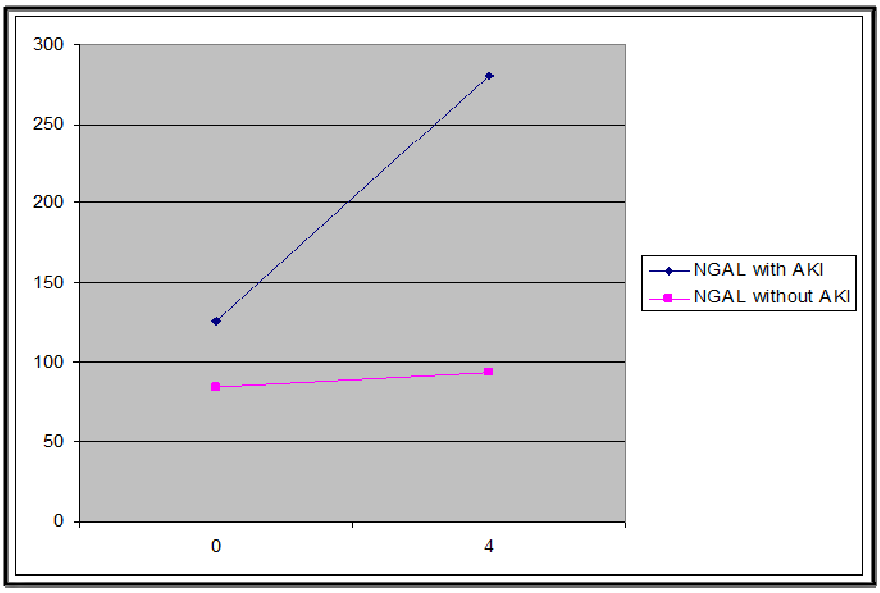

Figure 2
NGAL preoperative and postoperative (with & without AKI)

In this study, the mean plasma NGAL values measured preoperatively in the study group were higher than that of the mean plasma NGAL value of the controls. The increased mean plasma NGAL value in the study group preoperatively could be due to the associated comorbid conditions like advancing age, atherosclerosis, diabetes mellitus, hypertension, coronary artery disease, the use of exogenous toxins (contrast media, NSAIDS, aprotinin, ACE inhibitors), endogenous toxins (iron released from hemolysis) and the pro inflammatory state [1,14]. Studies have shown that NGAL levels were also elevated in metabolic and inflammatory diseases [15-17]. It was found that there was no difference in plasma NGAL levels between the patients who underwent CABG and the valve replacement surgeries. Cardio Pulmonary Bypass (on pump / off pump techniques) did not affect the plasma NGAL concentrations although it is considered to be one of the most important causes for AKI in patients undergoing cardiac surgery [18-20]. Plasma NGAL values at 4 hours after surgery were higher than the preoperative plasma NGAL values. Serum creatinine values at 48 hours and 72 hours after surgery were higher than that of the preoperative, 4 hours and 24 hours values. Mishra et al observed that both plasma and urinary NGAL levels were significantly increased ($p<0.001$) within 2-6 hours after surgery in children who developed AKI after elective cardiac surgery [21]. Wagener et al in 2006 showed that urinary NGAL levels were elevated within 1 hour and remained significantly higher at 3, 18 and 24 hours after cardiac surgery in adult patients who developed postoperative acute renal dysfunction [22]. Wagener et al in 2008 evaluated urinary NGAL levels after cardiac surgery in a larger patient population and showed that the urinary NGAL levels peaked immediately after cardiac surgery by more than 9 fold and remained significantly greater at 3, 18 and 24 hours after surgery[23]. In a study by Dent et al, the plasma NGAL at 2 hours after the surgery was observed to be the most powerful independent predictor of AKI and strongly correlated with the duration of AKI and the length of hospital stay in children undergoing cardiopulmonary bypass [24]. Tuladhar et al in 2009 demonstrated that both plasma and urinary NGAL levels were significantly increased at 2 hours after cardiac surgery in adult patients [25]. In a study done by Bennett M et al in 2008, NGAL was measured in urine samples of cardiac surgery patients by research ELISA and the ARCHITECT (standardized clinical platform) analyzers at periodic intervals after cardiopulmonary bypass observed that there was significant increase in urine NGAL postoperatively in children who developed AKI. The urine NGAL levels were increased 15 fold within 2 hours and by 25 fold at 4 and 6 hours after cardiopulmonary bypass [26]. Fadel FI et al observed that plasma NGAL levels could be used for the detection of AKI as early as 2 hours postoperatively and to confirm AKI at 12 hours with greatest accuracy and specificity in children who underwent cardiac surgery [27]. However many studies have shown that serum creatinine increases at 1-3 days after cardiac surgery [21, 22, 28-30]. The diagnosis of AKI is generally based on the changes in the creatinine levels or on the urine output based on either the RIFLE criteria or the AKIN criteria [1]. In this study AKI was diagnosed based on the AKIN definition i.e., an increase in serum creatinine concentration of ≥ 0.3 mg/dl developing over 48 hours. Out of the 53 patients who underwent surgery 13 developed AKI. They were classified under stage I of AKIN staging which is equivalent to the RISK staging of the RIFLE criteria. The serum creatinine levels for patients with AKI were greater than those without AKI. The serum creatinine levels were found to be elevated at 48 hours after surgery. The plasma NGAL levels were higher in patients with AKI than those without AKI. Plasma NGAL levels were elevated at 4 hours for AKI patients (as shown in figure 2). There was no significant elevation in patients without AKI, making NGAL a more reliable biomarker in comparison to creatinine. Of the patients who developed AKI none of them progressed to stage 2 which represents INJURY. Also adverse outcomes such as prolonged ICU stay, prolonged hospital stay, dialysis and death were not reported in any of them. This could be due to advances in post-operative management protocols, stringent patient monitoring techniques and round the clock availability of intensivists.

CONCLUSION

The significant elevation for creatinine occurs at 48 hours whereas the elevation is significant at 4 hours for NGAL making it an early predictor of AKI. Serum creatinine concentrations do not change until about 50% of kidney function has been lost and do not accurately depicts kidney function until a steady state has been reached. It usually increases 2-4 days after cardiac surgery. So serum creatinine is considered to be a delayed marker of AKI [8-11]. A number of new biomarkers that have been proposed and researched to predict AKI at an early stage include Cystatin C, Interkeukin-18 (IL-18), Kidney Injury Molecule -1 (KIM-1), Liver type Fatty Acid Binding Protein (L-FABP), N-Acetyl-D-Glucosaminidase (NAG) and Neutrophil Gelatinase Associated Lipocalin (NGAL)[31]. NGAL expression is markedly induced in injured epithelial cells including kidney, colon, liver and lung [32]. NGAL was identified as one of the most strikingly upregulated genes and over expressed proteins in the kidney after ischemia [33-35]. NGAL has been investigated in various clinical settings of AKI, such as after cardiac surgery, in critically ill patients, in contrast induced nephropathy, in delayed graft function of kidney transplantation and in emergency department. Not one biomarker but a collection of strategically selected proteins might provide the panel for early and rapid diagnosis of acute renal injury [36]. The present study identifies NGAL as a prime candidate for inclusion in such a panel. Such a method would be in dispensable for the timely institution of potentially effective treatments in early human acute kidney injury, a common clinical disorder still associated with a dismal prognosis for which intervention is desperately needed.

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CONFLICT OF INTEREST

Conflict of intertest declared none.

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