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Original Article

Plasma Neutrophil Gelatinase–associated Lipocalin (NGAL) and Plasma Cystatin C (CysC) as Biomarker of Acute Kidney Injury after Cardiac Surgery

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ABSTRACT. Acute kidney injury (AKI) is a common and serious condition that frequently occurs after cardiopulmonary bypass and other cardiac surgeries. The objective of this work is to evaluate the utility of new markers for kidney damage, plasma neutrophil gelatinase–associated lipocalin (NGAL) and plasma cystatin C (CysC) as early predictors of AKI after cardiac surgery. Fifty cardiac patients were recruited for this study, and they were divided into two groups of 25 patients each. Group I patients underwent coronary artery bypass graft (CABG) operation and Group II patients underwent valve replacement operation. Blood sample was taken for measurement of plasma CysC and NGAL by enzyme-linked immunosorbent assay. Plasma NGAL measurement in patients with AKI shows a highly significant rise at 3 and 6 h after surgery from its basal level (P <0.001). Plasma CysC measurement in patients with AKI is significantly higher at 6 h after surgery from its basal level (P <0.05). A statistically highly significant increase in plasma NGAL and CysC at 24 h after cardiac surgery in patients with AKI compared with patients without AKI (P <0.001). The sensitivity and specificity of NGAL at 3 h post-operative was 94.1% and 93.9% respectively, while plasma CysC sensitivity and specificity was 54.7% and 72.7%, respectively. After 6 h post-operative, NGAL sensitivity increased to 98.1% with slight decrease of the specificity to 91.9%, while CysC sensitivity and specificity increased to 75.2% and 75.8%, respectively. In conclusion, plasma NGAL and plasma CysC may be considered as early predictors of AKI after cardio-pulmonary bypass operations.

Introduction

Acute kidney injury (AKI) occurs frequently after cardiopulmonary bypass (CPB) and other cardiac surgeries, and is associated with increased morbidity, mortality and duration of intensive care treatment.[1] Often, AKI manifests as a transient rise in serum creatinine and is managed conservatively; however, a group of patients, often with significant comorbidity, require temporary renal replacement therapy.[2] The 30-day mortality of patients requiring dia-

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lysis after cardiac surgery is 60–80%. AKI after cardiac surgery is more common in individuals with pre-existing renal impairment and comorbidity. Clinical prediction tools have been developed to estimate the chance of AKI after cardiac surgery.[3] Serum creatinine, the currently accepted "gold standard" to diagnose AKI, is a delayed and inadequate marker of acute changes in renal function. In AKI, serum creatinine elevation that reflects the development and severity of kidney damage does not occur until days after renal tubular injury has begun.[4] Although serum creatinine is routinely used as a marker of renal function, it performs poorly in the immediate post-operative period. This is mainly due to hemodilution resulting from CPB.[5] This often results in a fall in serum creatinine even in the presence of significant renal injury. More importantly, serum creatinine usually rises only after 24–36 h of renal tubular damage and therefore does not fulfill the criteria for an early predictive biomarker of AKI. Thus, there is a need for rapidly available, sensitive and specific biomarkers for AKI that would allow early prediction at a time when intensive care optimization can be performed.[6] Recently, plasma cystatin C (CysC) was shown to detect AKI earlier than serum creatinine in critically ill patients. CysC is a non-glycosylated 13 kDa basic protein that is a member of the cystatin superfamily of cysteine protease inhibitors. It is produced by all nucleated cells, unaffected by muscle mass (unlike creatinine).[7] CysC is excreted by glomerular filtration, then undergoes essentially complete tubular reabsorption and catabolism (without secretion) so that it is not normally found in urine in significant amounts. This is in agreement with recent findings that demonstrate that elevated levels of urinary CysC may reflect tubular dysfunction and tubulointerstitial disease.[8] Neutrophil gelatinase–associated lipocalin (NGAL), a 25 kDa member of the lipocalin family, is markedly upregulated in the early post-ischemic mouse and rat kidney. NGAL levels in plasma and urine gets elevated earlier than serum creatinine in the setting of delayed graft function following kidney transplantation and percutaneous coronary intervention.[9] Mishra et al[10] demonstrated in children undergoing cardiac surgery that NGAL concentrations increase in plasma and urine within 2 h post-CPB surgery, preceding the serum creatinine elevation, in those who go on to develop AKI. There was no increase in children with stable perioperative renal function. Therefore, the aim of this work is to study whether plasma NGAL and plasma CysC could be early predictors of AKI after cardiac surgery.

Subjects and Methods

This work has been carried out in the Zagazig University Hospital from the period between June 2009 and June 2011 as a single-center prospective observational study on a total of 50 patients recruited for the study and classified into two main groups according to the types of cardiac operation. Group I included 25 patients who underwent coronary artery bypass graft operation. There were 18 males and seven females, with an age range from 48 to 60 years and mean values ± SD of 53.96 ± 3.64 years. They were all chosen with a negative history of diabetes, hypertension, renal diseases and malignancy. Their basal creatinine level was a mean value ± SD of 0.83 ± 0.4 mg/dL. Group II consisted of 25 patients who underwent valve replacement surgery. There were 14 males and 11 females, with an age range from 26 to 41 years and mean values ± SD of 34.8 ± 3.5 years. They were all chosen with a negative history of diabetes, hypertension, renal diseases and malignancy. Their basal creatinine value was 0.82 ± 0.3 mg/dL (mean ± SD). After the surgery, the patients were subdivided according to the development of AKI based on the RIFLE criteria into two other subgroups: AKI group, defined as the creatinine level at 24 h being elevated either by 25% of the basal level or by 0.3 mg/dL above the basal level. It included 17 patients (10 male and seven female) with an age range from 39 to 56 years (mean ± SD of 47.7 ± 8.8). No AKI group, defined as those with no rise of the serum creatinine level after 24 h of the operation. It included 33 patients (22 male and 11 female) with an age range

Table 1. Comparison of mean ± SD values of AKI biomarkers among the studied groups by an independent t-test.

	Coronary artery bypass graft (Group I) (mean ± SD)	**Valve replacement (Group II) (mean ± SD)**	**t-test**	***P***
Basal creatinine (mg/dL)	0.83 ± 0.4	0.82 ± 0.3	0.15	>0.05
Basal NGAL (ng/mL)	53.5 ± 8.5	53.2 ± 6.4	1.19	>0.05
Basal cystatin C (ng/mL)	2.64 ± 0.34	2.42 ± 0.25	1.7	>0.05
Duration of operation (min)	191.4 ± 33.7	90.2 ± 19.6	12.9	**<0.001**
AKI	12 (48%)	5 (20%)	4.3	**0.05**
NGAL: Neutophil Gelatinase- Assosiated Lipocalin , AKI: Acute Kidney injury				

from 32 to 53 years (mean ± SD of 42.6 ± 10.6).

Exclusion criteria

All subjects of this study were enrolled after obtaining their written consent. Those who were found to be free from hepatic disease, renal disease, malignancy, chronic infection, hypertension and diabetes mellitus were selected.

Methods

All subjects of the study were subjected to the following:

1) Full history and thorough clinical examination: According to the included work sheet with special stress on history of renal diseases
2) Measurements: All measurements were made according to the methods applied in the clinical pathology laboratories of the Zagazig University Hospitals.

Fasting blood and urine samples were collected for the routine and specific investigations. Urine analysis was performed for glucose, acetone, protein, pH, bilirubin and leukocytes by urine strips. Complete blood picture was carried out by an automated blood counter. Liver function tests were measured by the kinetic method.[11] Serum creatinine was performed by the method prescribed by Henry[12] and serum urea by the colorimetric method.[13] Calculation of glomerular filtration rate was done by using the MDRD equation:

$$GFR\ (mL/min/1.73\ m^2) = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742\ \text{if female}).$$[14]

Measurement of NGAL by ELISA

Biovendor NGAL is an enzyme immunoassay (ELISA) for the quantitative determination of NGAL levels in human plasma or serum.[15]

Measurement of CysC by ELISA

Biovendor CysC is an enzyme immunoassay (ELISA) for the quantitative determination of CysC levels in human plasma or serum.[16]

Collection of blood samples

Six milliliters of peripheral venous blood was taken from each subject under complete aseptic conditions. The samples were left for spontaneous clotting and then centrifuged at 3000 rpm for 5 min. Samples were separated and divided into three tubes for measurement of basal values of serum creatinine, serum NGAL and serum CysC. This process was repeated after 3, 6 and 24 h of the procedure.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Science version 10.0 for Windows (SPSS Inc., Chicago, IL, USA). The data were presented in the form of mean ± standard deviation (SD). Data were analyzed by an independent sample, paired t test and one-way analysis of variance (ANOVA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. *P*-value was considered significant if <0.05 and highly significant if <0.001.

Results

Table 1 shows the comparison of mean ± SD of AKI biomarkers among the studied groups by the independent t-test. No statistically signi-

Table 2. Time course of the studied markers among patients with AKI versus those without AKI.

Patients category	Biomarker	Basal	3 h	6 h	24 h	F	*P*
Patients with AKI	NGAL (ng/dL)	52.29 ± 4.49	95.41 ± 20.34[a]	127.05 ± 27.9[a,b]	91.35 ± 23.8[a]	58	<0.001
	Cystatin C (ng/dL)	2.44 ± 0.49	2.61 ± 0.48	2.71 ± 0.48[a]	3.2 ± 0.39[a,b]	33.04	<0.001
	Creatinine (mg/dL)	0.84 ± 0.06	0.84 ± 0.05	0.85 ± 0.09	1.15 ± 0.08[a]	69.75	<0.001
Patients without AKI	NGAL (ng/dL)	53.87 ± 11.21	58.06 ± 11.25	60.13 ± 11.24	59.15 ± 11.14	0.01	0.9
	Cystatin C (ng/dL)	2.36 ± 0.18	2.37 ± 0.19	2.38 ± 0.19	2.39 ± 0.18	0.01	0.9
	Creatinine (mg/dL)	0.81 ± 0.05	0.82 ± 0.07	0.82 ± 0.06	0.82 ± 0.09	0	1

[a]Significant from the base, [b]Significant from 3 h.

ficant difference was found in the basal mean ± SD values of creatinine, NGAL and CysC. However, statistically significant differences were found with regard to the duration of operation and number of AKI cases (P <0.05).

Table 2 shows the time course of the studied markers among patients with AKI versus patients without AKI. There were highly significant statistical differences along the time course for NGAL and CysC and a statistically significant difference along the time course for creatinine in patients with AKI. There was no statistical difference along the time course for NGAL and CysC and creatinine in patients without AKI.

Table 3 shows that there is a significant correlation between CysC and basal creatininie, basal NGAL level, MDRD and duration of the operation at 3 and 6 h post-operation. No significant correlation was found between NGAL and age and hemoglobin level at 3 and 6 h after the operation.

Table 4 shows the validity of the studied marker as predictors for AKI after 3 and 6 h. After 3 h, setting a cut-off value of 2.65 (ng/dL) for serum CysC yielded a sensitivity and specificity of 54.7% and 72.7%, respectively, and setting a cut-off value of 62 ng/dL for serum NGAL yielded a sensitivity and specificity of 94.1% and 93.9%, respectively; after 6 h, it yielded a sensitivity and specificity of 75.2% and 75.8%, respectively, for CysC and a sensitivity and specificity of 98.1% and 91.9%, respectively, for NGAL.

Discussion

AKI occurring after CPB and cardiac surgery is associated with increased morbidity, mortality and duration of intensive care treatment.[17] Often, AKI manifests as a transient rise in serum creatinine and is managed conservatively; however, the 30-day mortality of patients requiring dialysis after cardiac surgery is 60–80%.[2,18] However, serum creatinine is an inadequate marker for AKI.[19] More than 50%

Table 3. Correlation coefficient (r) value of serum Cystatin C (ng/mL) after 3 and 6 h versus some studied parameters in the AKI group.

	3 h		6 h	
	R	*P*	R	*P*
Age (years)	0.4	>0.05 NS	0.41	>0.05 NS
Basal creatinine (mg/dL)	0.46	<0.05 S	0.45	<0.05 S
Basal NGAL (ng/dL)	0.42	<0.05 S	0.44	<0.05 S
Hb (g/dL)	0.073	>0.05 NS	0.082	>0.05 NS
MDRD (mL/min)	-0.43	<0.05 S	-0.51	<0.05 S
Duration of operation (min)	0.45	<0.05 S	0.43	<0.05 S

Table 4. Validity of the studied markers as predictors for AKI 3 and 6 h post-operative.

	3 h post-operative	6 h post-operative
Cystatin (ng/dL)		
Cut-off value	2.65	2.65
Sensitivity (%)	54.7	75.2
Specificity (%)	72.7	75.8
PPV (%)	55	55.4
NPV (%)	80	81.8
NGAL (ng/dL)		
Cut-off value	62	62
Sensitivity (%)	94.1	98.1
Specificity (%)	93.9	91.9
PPV (%)	88.8	84.2
NPV (%)	96.8	96.7

of renal function must be lost before an elevation in serum creatinine is detected and serum creatinine does not accurately depict kidney function until a steady state has been reached. Although animal studies have shown that AKI can be prevented and/or treated using several maneuvers, these must be instituted very early after the insult. Therefore, we need other markers that would help us with early detection of AKI, and our findings show that monitoring of plasma NGAL levels can be of benefit to indicate an early warning to providers of critical care.

In this study, we observed that plasma NGAL measurement in patients with AKI showed a statistically significant rise at 3 and 6 h after surgery from the basal level: 52.29 ± 4.49 to 95.41 ± 20.34 and 127.05 ± 27.9, respectively, and a non-significant rise in patients without AKI. In keeping with our study, Tuladhar et al[20] found that there was a significant increase in NGAL concentration in all patients, and this increase in the levels post-cardiac surgery was greater in those patients who developed AKI compared with those who did not. Similar finding were also reported in the study of Dent et al,[21] who showed a significant rise of plasma NGAL at 3 h after CPB.

We also found that plasma CysC measurement in patients with AKI shows a non-significant rise at 3 h (2.44 ± 0.49 to 2.61 ± 0.48), which became significant at 6 h after surgery from a basal level of 2.44 ± 0.49 to 2.71 ± 0.48. This is similar to what was reported by Tuladhar et al[20] and Krawczeski et al,[22] who failed to find a significant rise in plasma CysC after 3 h.

In this study, a statistically highly significant increase in the mean value of serum NGAL and CysC at 24 h after cardiac surgery is found in patients with AKI compared with patients without AKI. Concurrently, we found that serum creatinine showed a statistically significant increase in patients with AKI compared with patients without AKI. These results go in harmony with the study carried out by Tuladhar et al[20] and Haase et al.[23]

However, conflicting results were reported by Royakkers et al regarding plasma CysC level, who found that it was a poor biomarker of AKI in the first 24 h after cardiac surgery.[24] This might be related to the low patient number (50 patients) in our study compared with 150 patients in their study, and the difference in the surgical technique.

The rapid rise at 3 and 6 h post-operatively and subsequent fall 24 h post-operatively in plasma NGAL (occurring while glomerular filtration is being lost) suggests that NGAL is likely to be a marker of cardiac surgery-associated tubular injury. These observations imply that tubular injury may be of even greater importance or greater proportional extent as indicated by the excellent predictive value of NGAL in this setting.[25]

An increase in creatinine may be measured only after a considerable loss of GFR, and this reflects an acute reduction of GFR for several days after the injurious event,[25] whereas CysC is an attractive marker for the assessment of

the GFR. It is synthesized by all nucleated cells and, unlike creatinine, which is produced by the muscle, it is not influenced by inter-individual differences and intra-individual changes in muscle mass.[26] Moreover, it is freely filtered by the glomerulus, completely reabsorbed by the proximal tubules and is not secreted by the renal tubules.[8] The rise in CysC concentrations in patients without a sCr-based diagnosis of AKI may indicate a sub-cohort of individuals in whom kidney injury occurred that was undetectable by conventional sCr-based criteria. Alternatively, plasma CysC may be affected by CPB-induced inflammation.[27]

CPB-associated AKI is mediated by renal tubular injury, whereas CysC is a marker of glomerular filtration rather than tubular damage *per se*. Glomerular filtration is impaired in setting of tubular injury because of tubular obstruction and tubulointerstitiual feed back and this may explain the the modest performance of plasma CysC as an early marker of AKI[23].[23]

In our study, we found that the sensitivity and specificity of NGAL at the 3 h post-operative period was 94.1% and 93.9%, respectively. It was high as compared with the sensitivity and specificity of 54.7% and 72.7%, respectively, for CysC. After 6 h, the NGAL sensitivity increased to 98.1% with a slight decrease of the specificity to 91.9%, which was associated with increased CysC sensitivity and specificity to 75.2% and 75.8%, respectively. Our study has certain limitations due to the relatively small sample size and, therefore, the predicted cut-offs for NGAL in plasma may be different from that obtained in other populations.

In conclusion, plasma NGAL and plasma CysC may be considered as early predictors of AKI after CPB operations. It allows the diagnosis of AKI up to 24 h prior to a clinical diagnosis based on conventional definitions of AKI. High risk patients may potentially have benefits from early therapies to be started before irreversible injury occurs

Conflict of interest: None.

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