PLASMA NEUTROPHIL GELATINASE–ASSOCIATED LIPOCALIN (NGAL) AND PLASMA CYSTATIN C (CYC) AS BIOMARKER OF ACUTE KIDNEY INJURY AFTER CARDIAC SURGERY

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Introduction: Acute kidney injury (AKI) occurs frequently after cardiopulmonary bypass (CPB) and cardiac surgery and is associated with increased morbidity, mortality, and duration of intensive care treatment**.**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**.** CyC 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. NGAL, a 25 kDa member of the lipocalin family, is markedly upregulated in the early postischemic mouse and rat kidney. Serum and urine NGAL levels are elevated earlier than serum creatinine in the setting of delayed graft function following kidney transplantation and percutaneous coronary intervention

Objective: the aim of work is to study if plasma NGAL and plasma Cystatin C could be an early predictors of AKI after cardiac surgery

Subject and methods: fifty patients were included in this study, they were divided into two main groups; Group(I) 25 patients underwent CABG operation ,Group(II) 25 patients underwent valve replacement operation then classified after the operation to AKI group and NO AKI group according to the RIFLE criteria.

Result: plasma NGAL measurement in patient with AKI shows a highly significant rise at 3and 6 hours after surgery from basal level. Plasma cysc measurement in patient with AKI shows an non significant rise at 3 hours that become significantly at 6 hours after surgery from basal level. a statistically highly significant increase in the mean value of plasma NGAL and cysc at 24 hour after cardiac surgery in patient with AKI compared with patients without AKI.serum creatinine show a statistical significant increase in patient with AKI compared with patient without AKI.the sensitivity and specificity of NGAL at 3h postoperative was 94.1% and 93.9% respectively. It was high in compared to plasma CysC 54.7% and 72.7 % respectively. After 6 hour postoperative; NGAL sensitivity increased to 98.1% with slight decrease of the specificity to 91.9% , which was associated with increased CysC sensitivity and specificity to 75.2% and 75.8% respectively.

Conclusion and Recommendations:-

In conclusions, plasma NGAL and plasma Cystatin C maybe considered as early predictors of AKI after cardio-pulmonary bypass operations. using plasma NGAL basally and 3houres after cardiopulmonary bypass as early renal biomarker of acute kidney injuery. using serum Cystatin C basally and 6houres after cardiopulmonary bypass as early renal biomarker of acute kidney injuery. Further studies are recommended using large number of patient**.**

INTRODUCTION

Acute kidney injury (AKI) occurs frequently after cardiopulmonary bypass (CPB) and cardiac surgery 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 dialysis after cardiac surgery is 60%–80%. AKI after cardiac surgery is more common in individuals with

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preexisting 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 postoperative 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 hours after renal tubular damage and therefore does not fulfil 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, serum CyC was shown to detect AKI earlier than serum creatinine in critically ill patients. CyC is a nonglycosylated 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**).

CyC 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 CyC may reflect tubular dysfunction and tubulointerstitial disease(**8**)

NGAL, a 25 kDa member of the lipocalin family, is markedly upregulated in the early postischemic mouse and rat kidney. Serum and urine NGAL levels are 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 serum and urine within 2 h postcardiopulmonary bypass, 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 was to study if plasma NGAL and plasma Cystatin C could be an early predictors of AKI after cardiac surgery

SUBJECTS AND METHODS

This work has been carried out in collaboration between the Internal Medicine , Cardiothoracic and Clinical pathology Departments, Faculty of Medicine, Zagazig University, during the period from June 2009 to June 2011.

* Subjects:

A total number of 50 patients were included and classified into two main groups:

1) Group I:

which includes 25 patients (18 male and 7 female) with age ranged from 48 years to 60 years with mean values + SD 53.96+ 3.64 years.they were all chosen with negative history of diabetes , hypertension, renal diseases and malignancy.Their basal creatinine level with mean values + SD of 0.83±0.4mg/dL

these patients underwent coronary arterybypass graft

2) Group II:

which includes 25 patients (14 male and 11 female) with age ranged from 26 years to 41 years with mean values + SD

34.8±3.5 years.they were all chosen with negative history of diabetes , hypertension, renal diseases and malignancy.Their basal creatinine level with mean values + SD of 0.82±0.3mg/dL

these patients underwent valve replacement surgery

the patients were classified after the proceduer into 2 groups :

AKI group : were the 24 h creatinine level was 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 7 female) with age ranged from 39 years to 56 years with mean values + SD 47.7±8.8 years. Their BMI ranges from 23 to 27 with mean value+SD 25.3+2.1.

No AKI group : no rise of the serum creatinine level after 24 h of the operation

It includes 33 patients (22 male and 11 female) with age ranged from 32 years to 53 years with mean values + SD 42.6±10.6 years. Their BMI ranges from 24 to 27 with mean value+SD 25.5+1.4.

Exclusion critieria :

All subjects of this study were enrolled after their written consent.They were selected to be free from hepatic,renal, malignancy,chronic infection , hypertension and diabetes melliteus

* Methods:

All subjects of the study were subjected to the following:-

A) Full history **and thorough clinical examination***:* According to the included work sheet with special stress on history of renal diseases

B) **Routine investigations***:*

They were all done according to the methods applied in the clinical pathology laboratories of zagazig university hospitals and include:

1- Urine analysis (for glucose, acetone, protein, pH, bilirubin and leukocytes by urine strips) **and stool analysis**.

2- Complete blood picture (by automated blood counter).

3- Fasting plasma glucose level

4- Liver function tests: serum bilirubin (total and direct), serum albumin, serum alanine transferase and aspartate transferase measured by kinetic method **(11)**.

5- Renal function tests: serum creatinine **(12)**, serum urea by colorimetric method **(13)**.

6- Calcuation of glomerular filtration rate using MDRD equation

GFR (mL/min/1.73 m2) = **175** x $(Scr)^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female)(**14**).

7- Lipid profile: included:
   a) Serum total cholesterol level by enzymatic colorimetric determination of total cholesterol *(15)*.
   b) Serum triglycerides by enzymatic method developed by **(16)**.
   c) HDL- cholesterol by method developed by **(17)**.
   d) LDL- cholesterol **(18)**.

8- Transthoracic echocardiography

C) Specific investigations: include

1-Meaurment of NGAL by ELISA:

BIOVENDOR NGAL is an Enzyme Immunoassay (ELISA) for the quantitative determination of Neutrophil Gelatinase Associated Lipocalin (NGAL) levels in human plasma or serum. **(19***)*

2- Meaurment of Cystatin C by ELISA:

BIOVENDOR Cystatin C is an Enzyme Immunoassay (ELISA) for the quantitative determination of Cystatin C levels in human plasma or serum. **(20)**

Collection of blood samples:

6 ml of peripheral venous blood were taken from each subject under complete aseptic conditions the samples were left for spontaneous clotting then centrifuged at 3000 rpm for 5 minutes. Samples were separated and divided into 3 tubes for measurement of basal values of serum

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creatinine, serum NGAL and serum Cystatin C. This process was repeated after 3 , 6 and 24 hours of the proceduer

Statistical analysis:-

Data were analyzed with SPSS version 15.0 (statistical package for the Social Science, Chicago, IL). Quantitative data were expressed as mean±standard deviation (SD) or standard error (SE). SE=SD/square root of patients number which was used in case of big SD, data were analyzed by independent sample, paired t test and one way analysis of variance (ANOVA). While qualitative data were expressed as number and percentage and were analyzed by Chi square (X2) test. Correlation was done using Pearson correlation test. The receiver operating characteristic (ROC) curve and 95% confidence interval (CI) was performed

to determine cutoff values for the studied biomarkers. 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 Comparison of Mean±SD of AKI Biomarkers among studied groups by independent t- test. No statistically significant difference was found in the mean values +SD among studied groups as regard basal creatinine (mg/dl) , basal NGAL (ng/dl), basal Cystatin C (ng/dl)**.** A statistically significant differences were found among studied groups as regard duration of operation and number of AKI cases (P<0.05)

Table (1): Comparison of Mean±SD of AKI Biomarkers among studied groups by independent t- test.

	CRONARY ARTERY BYPASS GRAFT (Group I) (Mean±SD)	VALVE REPLACEMENT (Group II) (Mean±SD)	t-test	P
BasalCreatinine (mg/dl)	0.83±0.4	0.82±0.3	0.15	NS
Basal NGAL(ng/ml)	53.5±8.5	53.2±6.4	1.19	NS
Basal cystatin C(ng/ml)	2.64±0.34	2.42±0.25	1.7	NS
Duration of operation (min)	191.4±33.7	90.2±19.6	12.9	HS
AKI	12(48%)	5(20%)	4.3	S

Table (2) : shows the time course of the stuided markers among patients with AKI versus without AKI. Thers was a highly statistical difference along the time course for NGAL (ng/dl) and Cystatin C (ng/dl) and a statisitical significant difference along the time course for

creatinine (ng/dl) in patient with AKI .There was no statistical difference along the time course for NGAL (ng/dl) and Cystatin C (ng/dl) and creatinine (ng/dl) in patient without AKI

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Table (2) : Time-course of the studied markers among patients with AKI versus those without AKI

Patients category	Biomarker	Basal	3hs	6hs	24hs	F	P
Patients with AKI	NGAL (ng/dl)	52.29±4.49	95.41±20.34	127.05±27.9	91.35±23.8	58	<0.001
	Cystatin C (ng/dl)	2.44±0.49	2.61±0.48	2.71±0.48	3.2±0.39	33.04	<0.001
	Creatinine (mg/dl)	0.84±0.06	0.84±0.05	0.85±0.09	1.15±0.08	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

Table (3) : shows the validity of the studied marker as predictors of AKI after 3 hours. Setting a cutoff value of 2.65 (ng/dl) for serum cystatin C yielded a sensitivity and specificity of 54.7% and 72.7% respectively with PPV of 55% and NPV of 80%. Setting a cutoff value of 62 (ng/dl) for serum NGAL yielded a sensitivity and specificity of 94.1% and 93.9% respectively with PPV of 88.8% and NPV of 96.8%

Table (3): Validity of of the studied markers as predictors for AKI 3hours postoperative.

Biomarker	Cutoff value	Sensitivity	specificity	PPV	NPV
Cystatin (ng/dl)	2.65	54.7%	72.7%	55%	80%
NGAL (ng/dl)	62	94.1%	93.9%	88.8%	96.8%

Table (4) : shows the validity of the studied marker as predictors of AKI after 6 hours. Setting a cutoff value of 2.65 (ng/dl) for serum cystatin C yielded a sensitivity and specificity of 75.2% and 75.8% respectively with PPV of 55.4% and NPV of 81.8%. Setting a cutoff value of 62 (ng/dl) for serum NGAL yielded a sensitivity and specificity of 98.1% and 91.9% respectively with PPV of 84.2% and NPV of 96.7%

Table (4): Validity of of the studied markers as predictors for AKI   6 hours postoperative.

Biomarker	Cutoff value	Sensitivity	specificity	PPV	NPV
Cystatin (ng/dl)	2.65	75.2%	75.8%	55.4%	81.8%
NGAL (ng/dl)	62	98.1%	91.9%	84.2%	96.7%

DISCUSSION

Acute kidney injury (AKI) occurs frequently after cardiopulmonary bypass (CPB) and cardiac surgery and is associated with increased morbidity, mortality, and duration of intensive care treatment. **(21).**

Often AKI manifests as a transient rise in serum creatinine and is managed conservatively; however, a group of patients, often with significant

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comorbidity, require temporary renal replacement therapy.(2)

The 30- day mortality of patients requiring dialysis after cardiac surgery is 60%–80%. AKI after cardiac surgery is more common in individuals with preexisting renal impairment and comorbidity. **(22)**

Clinical prediction tools have been developed to estimate the chance of AKI after cardiac surgery. These have identified female gender, impaired left ventricular function, insulin-requiring diabetes, emergency surgery, and abnormal baseline renal function as independent predictors of requirement for dialysis.(3)

So this work aimed to elucidate if plasma NGAL and Cystatin C could be considered as early markers of AKI before the beginning of serum creatinine to increase after cardiac surgery.

Our study included a total number of 50 patients divided into 2 groups. Group 1 included 25 patients that underwent coronary artery bypass graft and group 2 included 25 patients that underwent valve replacement surgery.

After the operation the patients were classified into 2 groups according to occurrence of AKI:

AKI group: included 17 patients who experienced an elevation of serum creatinine either by 25% of the basal level or by 0.3mg/dl above the basal level after 24 hours postoperatively

No AKI group: included 33 patients who didn't experienced an elevation of serum creatinine either by 25% of the basal level or by 0.3mg/dl above the basal level after 24 hours postoperatively

We observed in our study that plasma NGAL measurement in patient with AKI showed a highly significant rise at 3 and 6 hours after surgery from basal level (52.29 ± 4.49) to (95.41 ± 20.34) and (127.05 ± 27.9) respectively and non significant in patient with NO AKI group

In consistence with our study; **Tuladhar et al., (23)** found that there was a significant increase in NGAL concentration in all patients. This increase in the levels post cardiac surgery was greater in those patients who developed AKI compared with those who did not.

Similar finding also was reported in the study of **dent et al.,(24)** who showed a significant rise of plasma NGAL at 3 h after cardiopulmonary bypass.

We also found that plasma CysC measurement in patient with AKI shows an non significant rise at 3 hours ((2.44 ± 0.49 to 2.61 ± 0.48) that become significantly at 6 hours after surgery from basal level (2.44 ± 0.49 to 2.71 ± 0.48) .

This goes in agreement with **Tuladhar et al.,(23) and Krawczeski et al.,(25)** who failed to find a significant rise in plasma Cys C after 3 hours.

Our study found a statistically highly significant increase in the mean value of serum NGAL and CysC at 24 hour after cardiac surgery in patient with AKI compared with patients without AKI. Concurrently we found that serum creatinine showed a statistical significant increase in patient with AKI compared with patient without AKI. This results goes in harmony with the study of **Tuladhar et al.,(23) and Haase et al. (26).**

Regarding plasma CysC level conflicting results was reported by **Royakkers et al.,(27)** who found that it was a poor biomarker of AKI in the first 24 hour after cardiac surgery. This might be related to low patient number (50

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patients) in our study compared to 150 patients in their study and technique difference in operation.

The rapid rise at 3 and 6 hours postoperatively and subsequent fall after 24 hours postoperatively 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. **(28)**

An increase in creatinine may be measured only after a considerable loss of glomerular filtration rate, and reflects an acute reduction of glomerular filtration rate only several days after the injurious event.**(26).**

Plasma CysC is an attractive marker for the assessment of the GFR CysC is synthesized by all nucleated cells, and unlike creatinine, which is produced by muscle, it is not influenced by inter-individual differences and intra-individual changes in muscle mass.**(29)**

Moreover, CysC is freely filtered at the level of the glomerulus and completely metabolized at the level of the renal tubule without being secreted or reabsorbed. It is notable that although plasma CysC concentrations differed in patients with and without AKI at all time points postoperatively.**(8)**

The rise in plasma CysC concentrations in patients without a sCr-based diagnosis of AKI may indicate a subcohort 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.**(30).**

CPB-associated AKI is mediated by renal tubular injury, whereas CysC is a marker of glomerular filtration, rather than tubular damage per se.

In the setting of tubular injury, glomerular filtration eventually becomes impaired as a consequence of tubular obstruction and tubuloglomerular feedback and this lag may explain the modest performance of plasma CysC as an early marker of AKI.**(26).**

In our study; we found that the sensitivity and specificity of NGAL at 3h postoperative was 94.1% and 93.9% respectively. It was high in compared to plasma CysC 54.7% and 72.7 % respectively. After 6 hour postoperative; NGAL sensitivity increased to 98.1% with slight decrease of the specificity to 91.9% , which was associated with increased CysC sensitivity and specificity to 75.2% and 75.8% respectively.

This study demonstrates that plasma NGAL measurement in adult patients after cardiac surgery is an accurate predictor of subsequent renal dysfunction.

There is limitation to our study. Due to the relatively small sample size, the predicted cutoffs for NGAL in plasma may be different from that obtained in other populations.

In conclusions, plasma NGAL and plasma Cystatin C maybe considered as early predictors of AKI after cardio-pulmonary bypass operations.

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