SERUM NEUTROPHIL-GELATINASE ASSOCIATED LIPOCALIN AS A PREDICTOR OF POST-CONTRAST ACUTE KIDNEY INJURY IN CHILDREN

By

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ABSTRACT

Background: *Post-contrast acute kidney injury (PC-AKI) is a decrease in renal function that follows intravascular administration of contrast media. PC-AKI diagnosis using serum creatinine can be delayed. A new biomarker neutrophil gelatinase associated lipocalin (NGAL) is postulated to be more sensitive for patients prone to PC-AKI.*

Objective: *To assess serum NGAL changes as an early biomarker of PC-AKI in children with congenital heart disease undergoing cardiac catheterization.*

Methods: *This observational study included 30 children with congenital heart disease who underwent cardiac catheterization at the Pediatric Cardiac Catheterization Unit, New Children's Hospital, Ain Shams University during the period from September 2018 to June 2019. Serum NGAL was measured just before the catheterization, 6 hours and 24 hours after contrast media administration while serum creatinine was measured before and after 24 hours of contrast media administration.*

Results: *The median age was 30 months and 63% were females. Significant rise of serum NGAL was noted within 24 hrs after contrast administration ($p< 0.05$) while serum creatinine showed a non-significant rise ($p>0.05$). Serum NGAL was positively correlated with age, weight, height, body surface area and rate of contrast injection ($p< 0.01$). Higher levels of serum NGAL were found among patients who underwent diagnostic cardiac catheterization ($p<0.05$).*

Conclusions: *In children receiving CM for cardiac catheterization, serum NGAL was a sensitive biomarker that could detect PC-AKI as early as 6 hours. Possible risk factors for a raised NGAL include older age and diagnostic catheterization.*

Keywords: *post-contrast acute kidney injury, biomarker neutrophil gelatinase associated lipocalin, cardiac catheterization.*

INTRODUCTION

The term PC-AKI is used to describe a decrease in renal function that follows intravascular administration of contrast media. The decrease in renal functions is usually mild, and renal function usually returns to baseline values within 1-3 weeks. Like all forms of acute kidney injury (AKI), an episode of PC-AKI is a marker for increased short-and long-term morbidity and mortality and prolonged hospital stay **(van der Molen et. al., 2018)**.

Contrast media (CM) used in cardiac catheterization is a low osmolar non-ionic contrast media (Iohexol), which has less effect on cardiac function and fewer side effects than conventional ionic contrast media **(McDonald et. al., 2018)**.

A new biomarker for AKI is NGAL, a 25 kDa siderophore binding protein composed of 179 amino acids, a member of the Lipocalin family, covalently attached to human neutrophil gelatinase, secreted by activated neutrophils. NGAL is involved in processes of cell-mediated immunity, bacteriostatic effects, cell proliferation, differentiation and apoptosis processes. It increases when there are conditions such as infection and malignancy **(Lichosik et. al., 2015)**.

In the kidney, NGAL is mainly expressed in the loop of Henle and distal convoluted tubules. It’s filtered by the renal glomeruli and reversibly reabsorbed in the proximal convoluted tubules **(Andreucci et. al., 2016)**.

AIM OF THE STUDY

This work aimed to assess the changes in serum NGAL level which serve as an early biomarker of PC-AKI in children with acyanotic congenital heart disease undergoing cardiac catheterization.

PATIENTS AND METHODS

Ethical considerations:

Prior to conducting the study, the ethical approval of Ain Shams University ethical committee was obtained ensuring that the work complies with the principles of the Declaration of Helsinki in 1975.

Written informed consent was signed by caregivers before enrolment. All patients’ data were kept confidential and care givers had the right to keep them. No conflict of interests existed regarding the research or the

publications. No Funds were received to conduct the research.

Sample size:

The sample size estimation was done using the Epi Info7 program for sample size calculation, setting the confidence level at 95% and margins of error at 10% based on the work done by Padhy et. al (2014), a total of 30 patients was estimated to be sufficient sample size.

Patient population:

Inclusion criteria:

Patients with various congenital acyanotic heart disease aged 1 month to 10 years of both sexes. All of them were scheduled to undergo cardiac catheterization procedure in which contrast media injection was needed for angiography from September 2018 to June 2019.

Exclusion criteria:

1. Patients who received any vasoactive medication in the past week, during or within 72 hours post catheterization.
2. Patients who had congenital or acquired renal disorders.
3. Patients who had any concurrent infections.
4. Patients who had malignancy.
5. Patients who had any thyroid dysfunction.

Methods: This observational cohort study was carried out in Pediatric Cardiac Catheterization Unit, New Children's Hospital, Ain Shams University - Cairo; on 30 children who underwent cardiac catheterization. All patients were randomly selected.

All the studied children were subjected to:

I. **Full medical history** as well as full clinical examination just before enrollment in the study with special emphasis on previous or current cardiac and/or renal symptoms and signs.

II. **Laboratory evaluation:**

- Human serum NGAL) was measured by Enzyme Linked Immunosorbent assay (ELISA) using Human NGAL ELISA KIT by (cloud-clone, USA).
- Sample collection and preparation: three blood samples were withdrawn under complete aseptic conditions before the catheterization, 6hrs and 24hrs after CM administration into plain vacutainer tube and samples were clotted for 30 minutes before centrifugation for 15 minutes at 3000xg. The freshly prepared serum was stored in aliquots at -20 c until used for assaying serum NGAL.

- Normal serum NGAL level (median values ranging from 0.6 to 199 ng/ml). (Bchir et al., 2017)
- Serum creatinine (sCr) was measured at baseline and 24hrs after CM administration and was assayed on Beckman coulter AU 480 system by using modified Jaffe method (Beckman coulter, Inc.250s. Kraemer Blvd CA92821, USA).

III. Imaging studies:

- Echocardiography was performed using Vivid E9, (GE Vingmed, Horten, Norway) for assessment of cardiac functions and any associated structural abnormalities. It was performed by a single echocardiographer.

IV. Procedure:

- Cardiac catheterization via transfemoral approach was performed to all patients and a low osmolar non-ionic contrast media (Iohexol) was injected intra- arterial via catheters connected to power injector (Imaxeon-Avidia, Medrad, USA) with a pressure range from (400-800) psi and a rate range of 1-2 mL/kg/sec.

V. Postoperative follow up: After the procedure children were monitored for any postoperative complications clinically and by laboratory evaluation.

Statistical analysis:

Analysis of data was done using Statistical Package for Social Science (IBM SPSS) version 23 (Chicago, IL, USA). Quantitative variables were described in the form of mean, standard deviation (SD) and range when parametric. Meanwhile median and interquartile range (IQR) when data found to be non-parametric. Qualitative variables were described as number and percentages. The comparison between quantitative data and parametric in two groups were done by Independent t-test , while in two paired groups paired t-test was used and in more than two paired groups Repeated Measure ANOVA test was used. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

RESULTS

The results of the current study are displayed in the following tables:

Table (1): Descriptive data regarding demographic and anthropometric data

Demographic and anthropometric data		No. = 30
Age (months)	Median (IQR) Range	30 (12 - 72) 3 – 120
Sex	Female Male	19 (63.3%) 11 (36.7%)
Weight (Kg)	Mean ± SD Range	12.86 ± 7.45 5 – 35
Height (length) (cm)	Mean ± SD Range	89.20 ± 23.66 62 – 145
BSA (m²)	Mean ± SD Range	0.56 ± 0.23 0.3 – 1.18

BSA: body surface area

Table (2): Descriptive data regarding the type of cardiac catheterization

Cardiac catheterization	No. 30	Percentage (100%)
Diagnostic	8	27%
Interventional	22	73%
Types of interventional Cardiac catheterization		
PDA closure	10	33%
VSD closure	6	20.0%
Ballooning valvuloplasty	5	16.7%
Stent insertion for COA	1	3.3%

PDA: patent ductus arteriosus VSD: ventricular septal defect COA: coarctation of aorta

Table (3): Descriptive data of the contrast used.

Criteria and rate of contrast administration		
Contrast volume (mL)	Median (IQR) Range	36.7 (18 - 52) 10 – 105
Pressure of injector intra-arterial (psi)	Mean ± SD Range	630.0 ± 105.5 400 – 800
Rate (mL/sec)	Median (IQR) Range	12.17 (7 – 15) 6 – 35

psi: pounds per square inch

Table (4): Comparison between serum NGAL and creatinine levels at different assessment times

Different AKI biomarkers		Baseline No. = 30	6 hours No. = 30	24 hours No. = 30	Test value	P-value	Sig.
NGAL (ng/ml)	Mean ± SD	12.13 ± 5.04	13.41 ± 6.29	13.92 ± 6.21	**4.153•**	**0.022**	**S**
	Range	1 – 22.5	1.5 – 25	2 – 24			
Creat (mg/dl)	Mean ± SD	0.44 ± 0.07	-	0.44 ± 0.09	0.197 *	0.845	NS
	Range	0.3 – 0.5	-	0.3 – 0.6			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
*: Paired t- test; •: Repeated measure ANOVA test

The previous table showed that there was a statistically significant rise of serum NGAL from baseline at 6hrs and 24hrs while the rise in serum creatinine wasn't statistically significant.

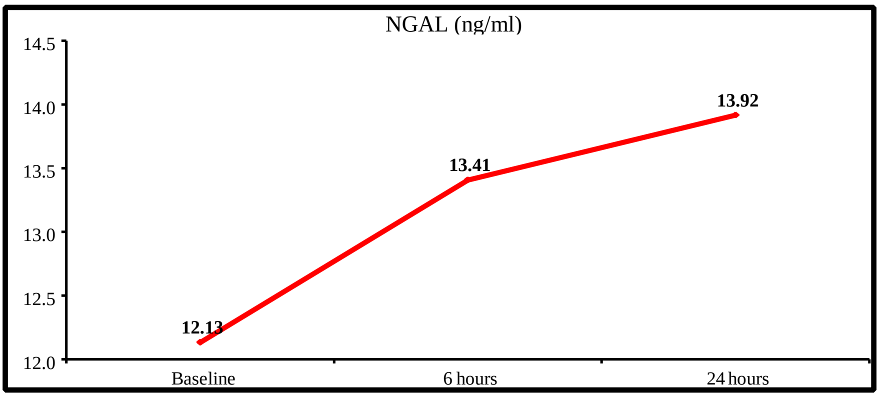

Figure (1): Diagram showing rising of serum NGAL from baseline till 24hrs.

Table (5): Correlation between serum NGAL at 24hrs and some study variables

Demographic, anthropometric data and laboratory data	24hrs		
	NGAL (ng/ml)		Sig.
	R	P-value	
Age (months)	**0.539****	**0.002**	**HS**
Weight (Kg)	**0.484****	**0.007**	**HS**
Height (Length) (cm)	**0.483****	**0.007**	**HS**
BSA (m^2)	**0.488****	**0.006**	**HS**
Volume of contrast	0.290	0.120	NS
Pressure of injector (psi)	0.222	0.239	NS
Rate of contrast (mL/sec)	**0.479****	**0.007**	**HS**
Creatinine (mg/dl)	0.303	0.103	NS

P-value >0.05: Non-significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
** Spearman correlation coefficients

Serum NGAL showed a highly significant positive correlation with age, weight, height (length), BSA and rate of contrast injection.

Table (6): Comparison between serum NGAL at 6hrs and 24hrs with different types of cardiac catheterization

Types of cardiac catheterization	NGAL 6hrs (ng/ml)		Pvalue	Sig.	NGAL 24hrs (ng/ml)		P-value	Sig.
	Mean	SD			Mean	SD		
Diagnostic	16.56	4.26	0.098	NS	18.44	3.99	**0.013**	**•S**
Interventional	12.26	6.59			12.27	6.11		

P-value >0.05: Non-significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: Independent t-test

The serum level of NGA in patients who underwent diagnostic cardiac catheterization, showed a gradual rise at 6 hrs which became statistically significant at 24hrs.

DISCUSSION

Post-contrast acute kidney injury (PC-AKI) is one of the significant complications of cardiac catheterization procedure. It was found that the incidence of PC-AKI ranging 4–10% following intravenous administration of contrast media **(Maloney et. al., 2019)**.

Serum creatinine increases only after marked decrease of glomerular filtration rate (GFR), its serum level increases after 24hrs. Recently a new promising biomarker NGAL is more sensitive than serum creatinine in early detection of PC-AKI **(Hwang et.al., 2014)**.

The aim of this study was to assess changes in serum NGAL level in children with acyanotic congenital heart disease undergoing cardiac catheterization at Ain Shams University New Children's Hospital Catheterization Unit as an early biomarker of PC-AKI.

The current study showed that 63.3% of the study population were females. Female sex predominance among pediatric patients was evident in many previous studies. In a study of **Lischosik et. al., 2015** they included 33 children aged from 1.5 months to 17 years, 58 % were females.

Interventional catheterization was done in 73% of patients compared to diagnostic cardiac catheterization (27%). The most frequent interventional procedure was PDA device closure accounting for 33%.

The interventional catheterization has now exceeded the diagnostic catheterization as the later was done in a small number of cases where the echocardiography wasn't able to delineate a certain anomaly **(Moustafa et al., 2016)**.

In this study, serum creatinine was monitored for the patients before and 24 hrs after contrast media administration with no statistically significant rise.

Hwang et. al., (2014) reached similar conclusion in their study of 26 children who underwent cardiac catheterization showed no significant increase in serum creatinine level within 24 hrs after cardiac catheterization. On the contrary, a study by **Tkaczyk et al., (2018)** on 50 children with congenital cardiac disease (64% were cyanotic heart disease) undergoing cardiac catheterization showed that serum creatinine fluctuated within the normal ranges for age but significantly increased within 24 hrs. It was observed that children who had cyanotic heart disease can be more prone to toxic agents due to chronic hypoxia leading to viscosity and increase in renal vascular resistance.

Serum creatinine was used as a biomarker to evaluate renal function but it has been reported that serum creatinine is not adequate for the prediction of precise renal outcome. Moreover, the level of serum creatinine can vary due to various extra-renal factors such as the amount of muscle and diet **(Mostafa et. al., 2016)**.

Newer kidney injury biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL) is a better indicator of kidney function compared to creatinine **(McDonald et. al., 2018)**.

Serum NGAL level of the studied patients showed a progressive rise from baseline over 24hrs. Similar to **Tomczyk et al., (2016)** who studied 16 children aged 7 days to 10 years old, rise in serum NGAL within 2 hrs after cardiac catheterization was noticed, but it was in hour 6 that the elevation was significant.

However, **Benzer et al. (2016)** a study of 91 patients (aged 3–19 years) who were scheduled for elective angiography stated that serum NGAL level showed a slight increase then gradually decreased when monitored after 12hrs and 24hrs successively.

Although NGAL is widely used for early diagnosis, monitoring and determining the prognosis of AKI patients, it is expressed at low concentrations in healthy human tissues such as trachea, lung, stomach, small intestine, and colon **(Negrin et. al., 2018)**.

In the present study serum NGAL was significantly positively correlated with age, weight, height and BSA. This was reported in many studies as that of **Sadat (2013)** and **Lischosik et al., (2015)** stated that other factors may influence NGAL concentration and hence interpretation of results should include factors such as age, gender and markers of inflammation.

Serum NGAL showed no significant correlation with the volume of contrast administered. Similarly, **Alharazy et al'S, (2014)** reported that the volume of contrast wasn't associated with development of AKI.

On the contrary, **Li et al. (2018)** noticed that patients who developed AKI received larger volume of contrast media than non-AKI patients.

It has been emphasized that the risk of PC-AKI is increased with repeated CM administration within a short interval (48-72 hrs). However, none of our patients had repeated CM administration. This could explain the non-significant correlation between serum NGAL and the volume of contrast administrated. In addition fluid administration increases urine flow rate, reduces CM concentration in the tubule and expedites CM elimination, thereby limiting exposure time of tubular cells to the deleterious effects of CM. **(Windpessl & Kronbichler 2019)**. All of our patients received fluids for proper hydration during preoperative and postoperative periods of fasting.

In the current study all patients received non-ionic, low osmolar (Iohexol). Similarly **Alharazy et al., (2014)** found that non-ionic, low osmolar contrast media wasn't a factor in the development of AKI.

Serum NGAL showed a highly significant positive correlation with the rate of contrast injection through an intra-arterial route. It was found that the rate of contrast media injection is associated with several complications. **Indrajit et al. (2015)**, **Moreno et al. (2013)**. In addition to intraarterial contrast media administration is more nephrotoxic than intravenous as it's closer to renal arteries. **Andreucci et al. (2014)**.

Serum NGAL showed a significant higher level among patients who underwent diagnostic cardiac catheterization in comparison to interventional cardiac catheterization. This observation is similar to that of **Tkaczyk et al. (2018)** who noticed that diagnostic cardiac catheterization with contrast media administration induced a significant rise of serum NGAL. This can be explained by the need for more contrast media administration during diagnostic cardiac catheterization for proper delineation of various anatomic structures during angiography in comparison to interventional cardiac catheterization which need little amounts of CM if any.

CONCLUSIONS

The present work recommends the implementation of the new biomarker serum NGAL as it potentially expands the possibility of renal function assessment as early as six hours post contrast administration among children undergoing cardiac catheterization. The raised NGAL suggests that other possible risk factors might precipitate PC-AKI namely diagnostic catheterization.

LIMITATIONS

The short period of follow up of patients (72 hours) and the wide variation in serum NGAL values among healthy subjects.

RECOMMENDATION

1. Early detection of PC-AKI should be a part of routine assessment of children post catheterization.
2. The ultimate need to a large multicenter study of the pediatric PC-AKI to provide guidelines for proper stratification of children at risk and hence better preventive measures could then be offered.

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