Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Plasma and Urinary NGAL-2 as Early Biomarkers for Diagnosis of Acute Kidney Injury in Pediatric Post-Operative Cardiac Patients

Waleed M. Serag[1*], Waleed M.El-Guindy[2], Sahar S. El-Sakka[1], Ebtsam S. Kotb[1], and Sherook Iprahim[3]
[1]Chemistry Department, Faculty of Science, Suez University
[2]Faculty of Medicine, Ain Shams University
[3] Central Blood Bank, Ministry of Health and Population , Suez

ARTICLE INFO

Keywords:
Acute kidney injury, Post-operative open cardiac surgery.
Neutrophil Gelatinase Associated Lipocalin (NGAL-2), Congenital heart disease

ABSTRACT

Acute kidney injury (AKI) is one of the critical multiples after pediatric cardiac surgery. The diagnosis of post-operative AKI is usually established based on elevated serum creatinine. Novel, effective and early diagnostic markers of AKI are still needed to be established. The aim of this study is validate new and faster biomarkers for early diagnosis of AKI. The survey population composed of 30 children aged from week to 204 months with a mean of months = 43.08 ± 52.15. These children were subjected to cardiac surgery with cardiopulmonary bypass (CPB).Then they were divided into AKI and non-AKI subgroups according to the post-operative status. Surveillance of the patients was achieved by subsequent measurements of both plasma and urinary NGAL, serum creatinine (S.Cr) and serum urea levels at 2 hours (h) and 24 h, postoperative. Additionally, only serum creatinine and serum urea were measured at 48 hours post-CPB. No significant differences between both groups

concerning the S.Cr levels at baseline (p=0.572,AUC=0.566), 2 h (p=0.619, AUC=0.558) and 24 h (p=0.197, AUC=0.651) while a high significant difference was observed at 48 h post-operative (p<0.001 , AUC=1.000) .No significant differences between the two groups were detected concerning the serum urea levels at baseline (p=0.455, AUC=0.587), 2 h (p=0.113 , AUC=0.685) and 24 h (p=0.129, AUC=0.677) while a high significant difference at 48 h (p<0.001 , AUC=1.000). There was no significant differences concerning the plasma and urinary NGAL levels measurements at baseline (p=0.215) while a highly statistically significant difference was spotted at 2 h (p<0.001) and at 24 h (p<0.001). Plasma and urinary NGAL serve as early predictors of AKI before the elevation of S.Cr. in postoperative patients.

Introduction:

The term “acute kidney injury” (AKI) is preferred to “acute renal failure” as it encompasses a wider range of renal impairment which can range from mild

**Corresponding author E-mail: waleed.ibrahim@suezuniv.edu.eg Telephone:01007893150*

impairment of renal functions and up to frank renal failure demanding renal substitution therapy[1]. It represents a frequent and a difficult to manage complication in post-operative patients of all ages, with persistently high rates of mortality and morbidity [2,3]. AKI prolongs hospital stays and elevates the mortality risk in those patients by three to nine times, depending on its severity [4]. AKI occurs in 36% of adult patients subjected to cardiac surgery [5]. The diagnosis of AKI is established based on the detection of raised serum creatinine which does not reflect changes of glomerular filtration rates in acute patients [6-7]. Many experimental studies have highlighted that although AKI can be reversed with various medications, however, the intervention should begin instantly after renal damage [8]. As such, it is obvious that for this intervention to be efficient, the treatment of AKI must begin as early as possible. New biomarkers of kidney damage are needed to ease early detection of kidney disease and establishing of suitable treatment, accordingly. These biomarkers should be able to discover early kidney damage as well as recognizing patients at a higher risk of progressive disease[9]. Among reported biomarkers, Neutrophil Gelatinase-Associated Lipocalin (NGAL or lipocalin-2) [10], a small molecule of 25-kDa belonging to a superfamily of proteins called lipocalins [11], is of great interest. Human NGAL composed of one disulphide-bridged polypeptide [12]. However the greatest of NGAL is in a monomeric shap, NGAL also happen as dimers and trimers, as well as in a compound with neutrophil gelatinase [12, 13]. The 25 kDa monomeric NGAL form is excreted by injured kidney tubule epithelial cells, whereas the dimeric form is the predominantly excreted by neutrophils [14, 15]. As its name implies, this protein was initially described in neutrophils [12], where it exerts strong antibacterial properties and under specific conditions may promote cellular apoptosis [9]. NGAL is also expressed at low grades in various tissues, including kidneys, lungs, stomach, and colon. NGAL expression is clearly stimulated in injured epithelia [8]. NGAL is maked systemically in reaction to kidney injury [9]. Increased regulation of NGAL expression is rapid, usually within 2 to 4 hours of injury. NGAL is also very stable and easily detected in urine [11]. The objective of the study is to evaluate the sensitivity and specificity of NGAL in plasma and urine (uNGAL), for the early diagnosis of acute kidney injury in patients subjected to open heart surgery.

Materials and Methods

Informed consent was obtained from patients caregivers and was confirmed by the Ethical Committee of Ain Shams University. All parents were assured about the confidentiality of the information gathered. Our survey was carried out in the Pediatric Cardiac Surgery Academy, Ain Shams University during the period from January 2017 to April2017 as a single-center observational survey on a total of 30 children aged week to 204 months with a mean of months 43.08 ± 52.15. Patients were subjected to open cardiac surgery with cardiopulmonary bypass for variable periods ranging from 21 up to 218 minutes depending on the type of lesion and the surgery done, which were either total correction or palliation of congenital heart disease. Patients with pre-existing renal impairment, peripheral vascular diseases, or on nephrotoxic drugs before surgery, as well as known diabetics were excluded from the study. Based on the development of postoperative AKI, the subjects were divided into AKI group and non-AKI

group. The AKI group included 9 (30%) patients [7 (23.3%) were males and 2 (6.7%) were females] with age ranging from 0.25 to 108 months with a mean 18.81 ± 34.03 SD of months, while the non-AKI group included 21 (70%) patients [12 (40%) were males and 9 (30%) were females] with age ranging from 3.0 to 204 months and the mean was 53.48 ±55.70 SD of months. Plasma and urinary NGAL by enzyme-linked immune sorbent assay (ELISA) as well as traditional serum creatinine and serum urea were done to all patients included in this study. Surveillance of the patients was achieved by various measurements of all these markers levels at 2 h and 24 h, postoperative. Additionally, postoperative serum creatinine and serum urea were measured at 48 h post-CPB. Estimation of plasma and urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) were carried out using Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) ELISA kit(Catalog #: 95645)of Glory Science Co., Ltd; USA.It was measured on MR-96 Microplate Reader (CLINDIAG SYSTEMS B.V.B.A; MR-96; MR2GL002; Belgium). Estimation of serum creatinine was carried out using commercially available reagent kit. The diagnostic kit of the kinetic Jaffé method used for the estimation of serum creatinine was obtained from Egyptian Company for Biotechnology (S.A.E). It was measured on JENWAY 7300 spectrophotometer (Bibby Scientific Ltd , Dunmow, Essex.CM6 3LB; 7300; UK). Estimation of serum urea was carried out by urease-colorimetric method (Modified Urease-Berthlot Method). The diagnostic kit used for the determination of serum urea was obtained from Egyptian Company for Biotechnology (S.A.E). It was measured on JENWAY 7300 spectrophotometer (Bibby Scientific Ltd, Dunmow, Essex.CM6 3LB; 7300; UK).

Statistical Analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 22.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test, and D'Agstino test, also Histogram and QQ plot were used for a vision test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, the comparison between the two studied groups was done using independent t-test while for abnormally distributed data comparison were done using Mann-Whitney test. We evaluated the sensitivity and specificity of each marker using Receiver Operating Characteristic (ROC) curves. The area under the ROC curve was used to quantify the ability of biomarkers to predict postoperative AKI. We used Pearson's correlation coefficients to analyze the relationship between the different variables. For all tests, a probability $p < 0.05$ was considered significant and $p < 0.001$ was considered highly significant.

Results:

During the study there were 19 males (63.3%) and 11 females (36.7%), mean age was 43.08 months, mean weight was 16.32 kg, mean length 91.23 cm, and mean BMI was 16.15 of kg/m^2 (Table 1).

Patients with congenital heart disease were divided into two categories: cyanotic group that included 21 patients (70%) and acyanotic group that included 9 patients (30%). In our study, AKI occurred exclusively in patients with cyanotic heart diseases. (Tables 2) ,(figure 1). A statistically significant was noted between the two groups with respect to the type of congenital heart defect (p=0.029).

Regarding serum creatinine, there was no significant difference between baseline and immediate postoperative values ***(t*** = 0.714, p = 0.481*)*. Although there was a rise in serum creatinine postoperatively, yet values at 2 h and 24 h postoperative were not significantly difference from each other as well as from preoperative values *(*t = 0.615, p = 0.544at 2 h*)* and *(*t =1.621, p = 0.116at24 h). On the contrary a highly significant difference was found at 48 h *(*t = 17.146, p < 0.001*)* (Table 3),(figure 2). Similarly, no significant differences were spotted between both groups concerning serum urea at baseline (t = 1.698, p = 0.124) at 2 h (t = 1.605, p = 0.143) at 24 h (t =1.420, p = 0.189)(Table 4), (figure 3).

Regarding Plasma and Urinary NGAL, no significant differences were spotted between the two groups preoperatively (t = 1.256, p = 0.220)and(t = 0.595, p = 0.557). On the other hand, statistically significant differences between the 2 groups were found *(*t = 29.915, p < 0.001*), (*t = 39.533, p < 0.001*)*at 2 h and (t = 25.294, p< 0.001), (t = 59.971, p < 0.001*)* at 24 h (Table 5), (figure 4, 5).

As shown in all Tables and figures, Plasma and Urinary NGAL showed significant rise at 2 hpostoperative before significant changes in serum creatinine and urea were detected.

Discussion

S.Cr. is an unsuitable sign for AKI [8] due to its drawbacks such as; (i) More than 50% of renal function must be missed before a rising in serum creatinine is discover. (ii) It does not accurately depict kidney function to a fixed state has been reached. However, NGAL offers a good sign for AKI as it collects within two distinct pools, namely a systemic and a renal pool. Interestingly that AKI results in a rising NGAL mRNA expression in distant organs, especially the liver and spleen, and the over-expressed NGAL protein is extreme likely emitted into the circulation and constitutes the systemic pool [16, 17]. Additional contributions to the systemic pool in AKI may be obtained from the truth that NGAL is a known acute stage reactant and may be emitted from neutrophils, macrophages, and other immune cells [18]. Furthermore, any decline in glomerular filtration rate producing from AKI would be predictable to decline the clearance of NGAL with further collection in the systemic pool. Gene expression studies in AKI have also shown fast thick ascending limb of Henle's loop and the collecting ducts, with a resultant synthesis of NGAL protein in the distal nephron (the renal pool) and excretion into the urine where it comprises the main portion of urinary NGAL [16,17]. NGAL in the urine and/or plasma were set to be early predictive biomarkers of AKI in cardiopulmonary bypass [19]. However, subsequent studies have demonstrated that the utility of plasma NGAL measurements is not restricted only to the CPB population. For

example, plasma NGAL is also an early, sensitive, specific, and predictive biomarker of AKI after contrast administration [20] and clearly indicates that plasma NGAL is a powerful early biomarker of AKI that precedes the increase in serum creatinine by several hours to days. The existing survey was carried out using pediatric patients that underwent cardiac surgery with extracorporeal circulation, and showed that NGAL is useful both in serum and urine samples for the early (in the first few hours following the surgical operation) detection of patients that will develop AKI in the coming days, with extremely high sensitivity and specificity levels. The usefulness of NGAL (in blood or urine samples) for the early detection of AKI following cardiac surgery was later confirmed in both children[21, 22] and adults [4,23-27], although the results are more diverse and are clearly worse in adults. In other studies, however, NGAL was predictive for AKI in urine samples, but not in serum,[24,28] and some studies have even produced negative results from using urine NGAL [29]. Our study demonstrates that in a large cohort of patients undergoing general surgery, immediate postoperative plasma and urinary NGAL levels were significantly more elevated in patients who went on to have AKI compared to those with Non-AKI. Plasma and Urinary NGAL were able to predict AKI, indicating that its levels correlated with severe renal injury days before S.Cr increases.

Conclusion

We have demonstrated that in patients undergoing CPB surgery, the incidence of S.Cr elevation is low, postoperative Plasma and Urinary NGAL concentrations predicted the development of AKI. Plasma and Urinary NGAL measurements may, therefore, facilitate the rapid diagnosis of AKI and distinguish it from Non-AKI. Plasma and urinary NGAL are early predictive biomarkers of AKI, morbidity, and mortality after pediatric CPB. Data from the present survey propose that plasma and urinary NGAL serve well in predicting AKI before the elevation in S.Cr. becomes apparent and who will have persistent AKI.

Ethical Consideration:

An informed agreement was passable from parents of all sharers. Each parent has the truth to accept or refuse participation after explaining the aim. Confidentiality of collected datum was secured to sharers. The survey was approved by ethical committee of pediatric department, Faculty of Medicine, Ain Shams University, that is organized and operated according to the Declaration of Helsinki for the human subject researcher.

Authors' contributions :

WMS controlled the experimental analysis, collected the scientific data, wrote and performed the first revision for the article.WME performed the medical supervision of the pediatric cardiac patients in the pre and post-operative status.SSE performed the final revision of the article and adjustment of the references. ESK and SI collected the samples, obtaining and interpretation of the results and performed Statistical Analysis. All authors read and approved the final manuscript.

Abbreviations list

AKI: Acute kidney injury
NGAL: Neutrophil Gelatinase-Associated Lipocalin
uNGAL: urinary Neutrophil Gelatinase-Associated Lipocalin
S.Cr: serum creatinine
ELISA: enzyme-linked immune sorbent assay

References

1. **Cruz DN, de Cal M, Garzotto F, Perazella MA, Lentini P, Corradi V, Piccinni P, Ronco C (2010):** Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive care med* **36(3)**444-451.
2. **Goldstein SL(2006):** Pediatric acute kidney injury: it's time for real progress. *Pediatr Nephro* **21(7)**891-895.
3. **Nguyen MT, Devarajan P (2008):** Biomarkers for the early detection of acute kidney injury.*Pediatr Nephro*, **23**(12):2151-2157.
4. **Han WK, Wagener G, Zhu Y, Wang S, Lee HT (2009):** Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery.*Clin JAm SocNephro***, 4(5)**873-882.
5. **Bagshaw SM, George C, Dinu I, Bellomo R(2008):** A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephro Dial Transplant* **23(4)**1203-1210.
6. **Ostermann M, Chang RW(2007):** Acute kidney injury in the intensive care unit according to RIFLE.*Crit care med.* **35(8)**1837-1843.
7. **Stevens LA, Coresh J, Greene T, Levey AS (2006):** Assessing kidney function—measured and estimated glomerular filtration rate. *New England J. Med* **354(23)**2473-2483.
8. **Devarajan P (2007):** Emerging biomarkers of acute kidney injury. In: *Acute Kidney Injury. Volume 156*, edn.: Karger Publishers. 203-212.
9. **Gharishvandi F, Kazerouni F, Ghanei E, Rahimipour A, Amirrsouli H, Nasiri M (2013):** Assessment of Neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker for detection of renal impairment in hypertensive patients.*J. Paramed Sci* **5**(1).
10. **Flower DR, North AC, Sansom CE (2000):**The lipocalin protein family: structural and sequence overview.*Biochim Biophy Acta (BBA)-Protein Struct. Mol Enzym* **1482(1)**9-24.
11. **Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M(2008) :** Neutrophil gelatinase–associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis* **52(3)**595-605.
12. **Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N (1993)**: Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *JBiolog Chem* **268**(14)10425-10432.
13. **Yan L, Borregaard N, Kjeldsen L, Moses MA (2001)** The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL) modulation of MMP-9 activity by NGAL.*J. Biolog Chem* **276(40)**37258-37265.
14. **Cai L, Rubin J, Han W, Venge P, Xu S (2010):** The origin of multiple molecular forms in urine of HNL/NGAL. *Clin J Am Soc Nephro.* **5(12)**2229-2235.
15. **Mårtensson J, Xu S, Bell M, Martling C-R, Venge P (2012):** Immunoassays distinguishing between HNL/NGAL released in urine from kidney epithelial cells and neutrophils. *Clin Chim. Acta* **413(19)**1661-1667.
16. **Schmidt-Ott KM, Mori K, Kalandadze A, Li J-Y, Paragas N, Nicholas T, Devarajan P, Barasch J (2006):** Neutrophil gelatinase-associated lipocalin-

mediated iron traffic in kidney epithelia. *Curr.Opin.Nephro Hyperten* **15(4)**442-449.

17. **Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, Barasch J (2007):** Dual action of neutrophil gelatinase–asso(1995). Identification of a new acute phase protein.*J Biolog Chem* **270(38)**22565-22570.

18**.Devarajan P (2010)** . Neutrophil gelatinase-associated lipocalin: A troponin-like biomarker for human acute kidney injury, Nephrology, Volume 15, Issue 4 June 2010 Pages 419–428

19. **Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J (2005):** Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery.*The Lancet* **365(9466)**1231-1238.
20. **Hirsch R, Dent C, Pfriem H, Allen J, Beekman III RH, Ma Q, Dastrala S, Bennett M, Mitsnefes M, Devarajan P (2007):** NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephro* **22(12)**2089-2095.
21. **Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J, Devarajan P (2007):** Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Critical care* **11(6)**R127.
22. **Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, Syed H, Ali S, Barasch J, Devarajan P (2008): Urine NGAL** predicts severity of acute kidney injury after cardiac surgery: a prospective study.*Clin J Am Soc Nephro* **3(3)**665-673.
23. **Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT (2008):** Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery.*Am J Kidney Dis* **52(3)**425-433.
24. **Xin C, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M (2008):** Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. *Renal failure* **30(9)**904-913.
25. **Matalanis G, Dragun D, Haase M(2009):** Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. *Crit Care Med*, **37(2)**.
26. **Tuladhar S, Puntmann V, Soni M, Punjabi P, Bogle R (2009):** Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass.*J Cardiovas.Pharmaco* 53(3)261.
27. **McIlroy DR, Wagener G, Lee HT (2010):** Neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery: the effect of baseline renal function on diagnostic performance.*Clin J AmSoc Nephro* **5(2)**211-219.
28. **Koyner JL, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V, Kasza KE, FO'Connor M, Konczal DJ, Trevino S (2008):** Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. *Kidney inter* **74(8)**1059-1069.
29. **Liangos O, Tighiouart H, Perianayagam MC, Kolyada A, Han WK, Wald R, Bonventre JV, Jaber BL (2009):**

Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass.*Biomarkers* **14(6)**423-431.

Table 1: Demographic data of patients[a]

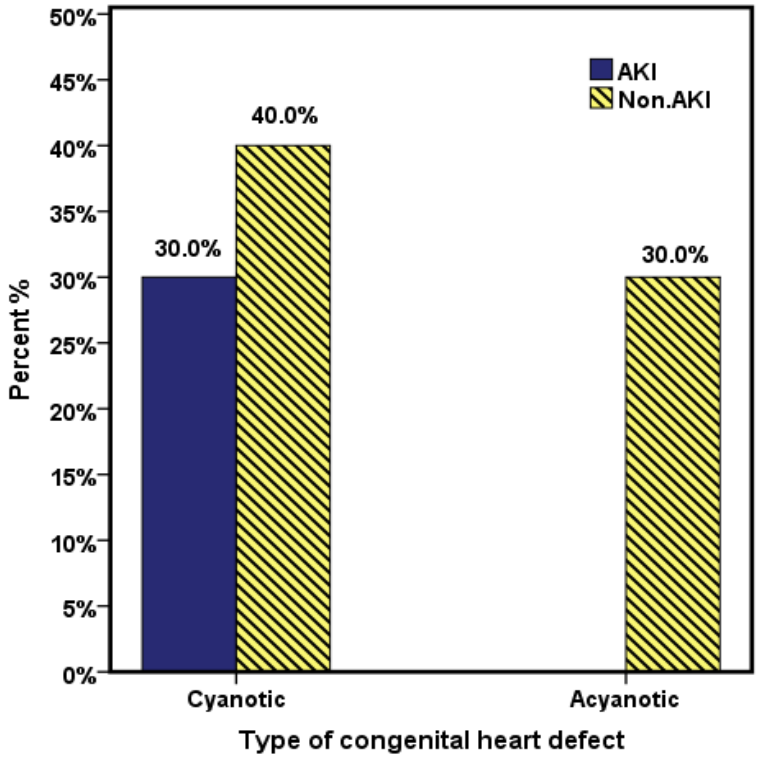
Patient characteristics	Total	
	No.	%
Sex		
Male	19	63.3
Female	11	36.7
Age (months)		
Min. – Max.	0.25 – 204.0	
Mean ± SD	43.08 ± 52.15	
Weight (kg)		
Min. – Max.	3.00 – 83.00	
Mean ± SD	16.32 ± 16.19	
Length (Cm)		
Min. – Max.	49.00 – 180.00	
Mean ± SD	91.23 ± 33.045	
BMI (kg/m^2)		
Min. – Max.	12.0 – 25.62	
Mean ± SD	16.15 ± 2.52	

[a]Total number of patients = 30 children

Table 2 : Comparison between AKI and non-AKI cases according to the type of congenital heart defect in total patients.

Type of congenital heart defect	Total (n = 30)		AKI (n = 9)		Non-AKI (n = 21)		χ^2	^{FE}p
	No.	%	No.	%	No.	%		
Cyanotic	21	70.0%	9	30.0 %	12	40.0%	5.510	**0.029 (S)**
Acyanotic	9	30.0%	0	0.0%	9	30.0%		

Fig. 1:Comparison between the two studied groups according to the type of congenital heart defect.



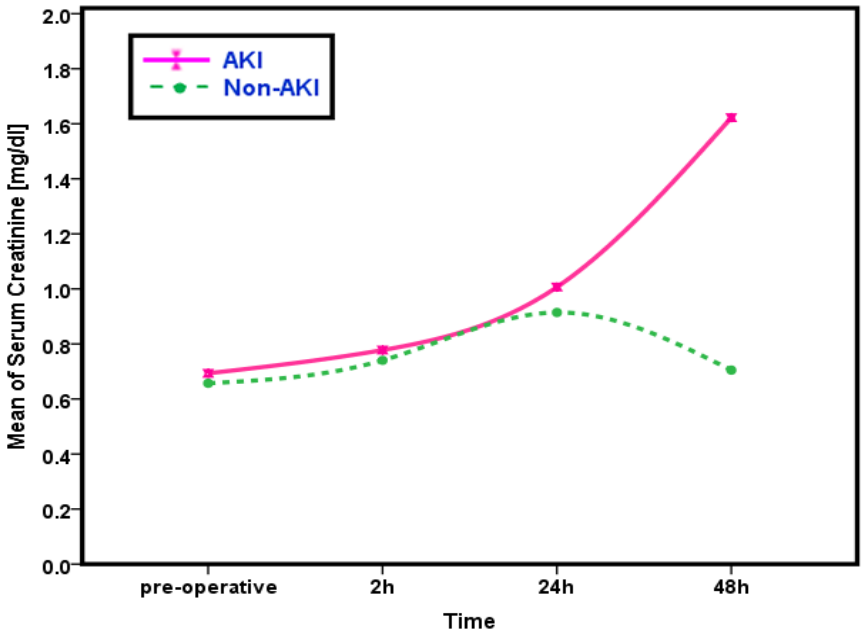


Fig. 2: Comparison between AKI and non-AKI cases according to S.Cr levels.

Table 4: Comparison between AKI and non-AKI cases according to serum urea levels in total patients:

	Baseline	2 hours	24 hours	48 hours

AKI(urea , mg/dl)				
Min. – Max.	17.00–36.00	25.00–45.00	32.00–45.00	53.00–56.00
Mean ± SD.	28.78±7.81	34.78±7.33	38.00±3.50	54.22±1.09
Non AKI (urea, mg/dl)				
Min. – Max.	10.00–39.00	13.00–40.00	24.00–45.00	17.00–40.00
Mean ± SD.	26.91±7.33	29.14±7.55	34.05 ±6.30	28.095±5.53
t	0.630	1.889	1.757	20.724
P-value	0.534 **(NS)**	0.069 **(NS)**	0.090 **(NS)**	**<0.001 (HS)**

t: Student t-test; NS: No statistically significant; HS: high statistically significant at $p \leq 0.001$

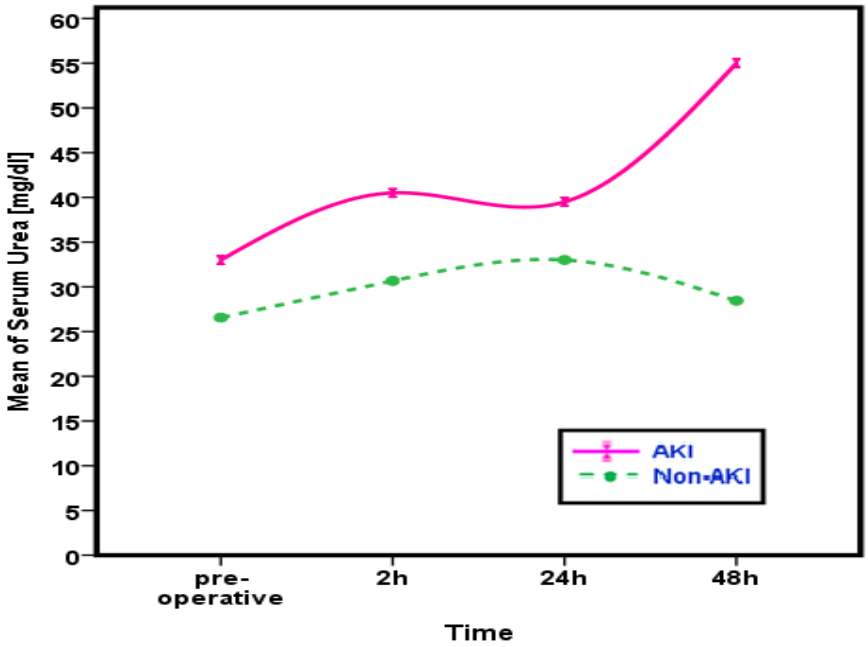


Fig. 3: Comparison between AKI and non-AKI patients according to Serum urea levels.

Table 5: Comparison between AKI and non-AKI cases according to plasma and urinary NGAL levels in total patients

	Baseline	**2 hours**	**24 hours**
AKI (Plasma NGAL ,ng/ml)			
Min. – Max.	1.96 – 2.84	27.97 – 35.71	32.91– 44.55
Mean ± SD.	2.36 ± 0.35	31.37 ± 2.73	38.06 ± 4.13
AKI (Urinary NGAL ,ng/ml)			
Min. – Max.	0.82 – 1.17	25.41 – 35.91	46.72– 57.19
Mean ± SD.	0.95 ± 0.13	31.46 ± 3.47	50.85 ± 3.64
Non-AKI(Plasma)			
Min. – Max.	1.92 – 2.78	2.46 – 4.95	1.92 – 4.09
Mean ± SD.	2.22 ± 0.25	3.89 ± 0.63	3.10 ± 0.63
Non AKI(Urine)			

Min. – Max.	0.82 – 1.25	1.01 – 2.88	1.7 – 3.92
Mean ± SD.	0.98 ± 0.12	1.73 ± 0.43	2.59 ± 0.65
t (plasma/urine)	1.256/0.595	29.915/ 39.533	25.294/59.971
P-value (plasma/urine)	0.220/0.557 **(NS) / (NS)**	<0.001/<0.001 **(HS)/(HS)**	<0.001/<0.001 **(HS)/(HS)**

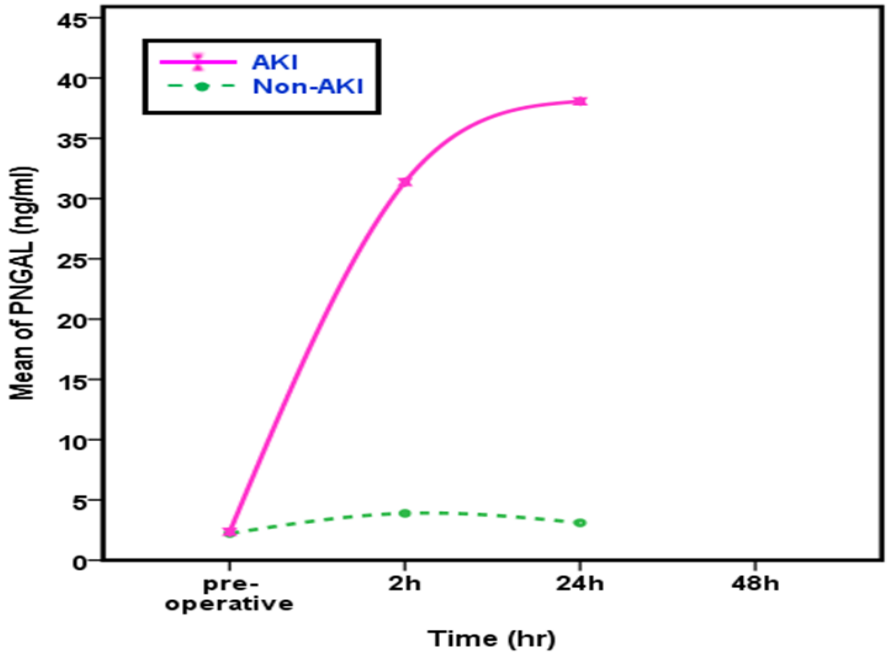


Fig. 4: Comparison between AKI and non-AKI cases according to plasma NGAL levels.

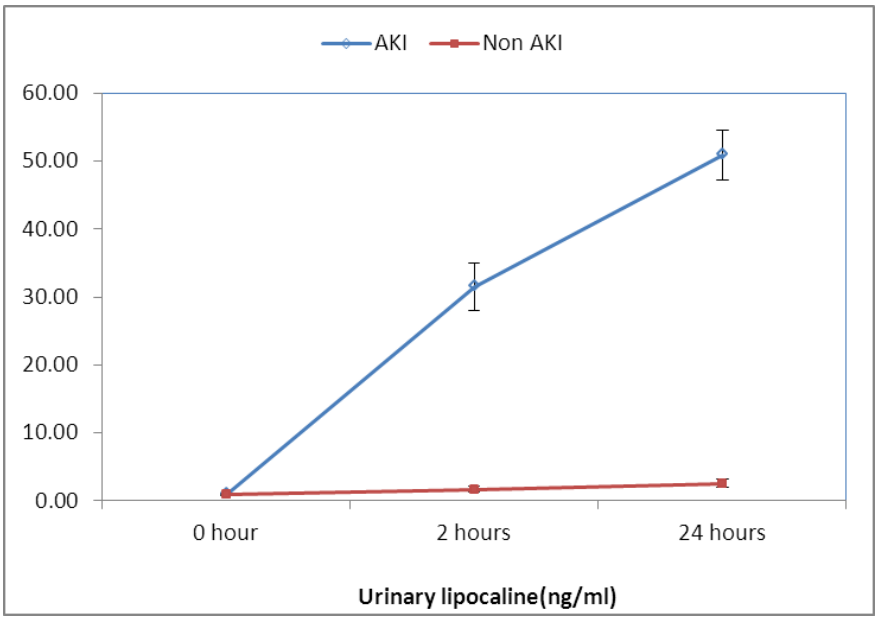


Fig. 5: Comparison between AKI and non-AKI cases according to urinary NGAL levels.