Original Article

Acute Kidney Injury among Post Cardiac Surgery Patients : A Retrospective Study

Sunil C V S Mutyala[1], Jagadeesh N Vaggar[2], Shirish Borker[3], Sushmit Kamat[4], Shreya S Lotliker[5], Jagadish Cacodcar[6], Jagannath P Kolwalkar[7], Wilroy Anthony Gonsalves[8]

Background : Acute Kidney Injury (AKI) is a common complication Post Cardiac Surgery with reported incidence of 20-70%. Various studies have been conducted worldwide on risk factors contributing to the etiology of AKI in Cardiac surgery patients. We undertook similar study to understand the etiology and risk factors associated with AKI at Goa Medical College hence we undertook this study.

Methodology : A retrospective record based observational study was conducted at Goa Medical College; wherein records of 419 patients who underwent Cardiac Surgery during the study period were analyzed for pre-operative, intra-operative and postoperative variables. Kidney Disease Improving Global Outcomes criteria were used to study the incidence of AKI. The Data was entered in Microsoft Excel and analysed using SPSS version 22.0. Chi-square test and Student t test were used as a test of significance.

Results : Out of 419 patient records reviewed; 40.3% patients developed AKI after Cardiac Surgery. Age, Sex, h/o previous Cardiac Surgery, CPB duration, Aortic Cross Clamp Time, addition of vasopressor etc. were some of the significant risk factors associated. AKI associated with Cardiac Surgery was associated with a mortality of 8.3%. Mean duration of ventilation 38.48±62.27 hrs. and ICU stay 6.12±3.15 days was comparatively longer than patients without AKI (P<0.001).

Conclusion : We concur that AKI is a serious complication in patients undergoing Cardiac Surgery and has significant impact on the outcome of the patients in terms of duration of ICU stay, duration of ventilation and mortality. There is need to identify modifiable risk factors at the earliest and develop approaches to improve the outcome and decrease the AKI associated morbidity and mortality.

[*J Indian Med Assoc* 2023; **121(3):** 15-20]

Key words **: Acute Kidney Injury (AKI), Cardiac Surgery, Cardiopulmonary Bypass.**

Acute Kidney Injury (AKI) is defined as an abrupt decrease in kidney function, which encompasses both injury (structural damage) and impairment (loss of function)[1]. Classification of AKI includes pre-renal AKI, Postrenal obstructive nephropathy and intrinsic Acute Kidney Disease. As the pre-renal and Postrenal causes persist they progress to renal cellular damage[1]. AKI manifest from mild to serious Renal Derangement /Failure if preventive and reparative measures are not taken in time.

AKI is a common complication Post Cardiac Surgery which carries prolonged morbidity and increased mortality. The reported incidence of AKI varies from 20-70 %[2]. The mortality rate is around 40-70% among patients undergoing Cardiac Surgery who needed Renal Replacement Therapy (RRT)[2]. Also, the

Editor's Comment :

- Acute Kidney Injury has significant impact on the outcome of the patients undergoing cardiac surgery
- Prevention of modifiable risk factors and early diagnosis is the key to decrease mortality and morbidity associated with Acute Kidney Injury.
- There is need to find out biomarker/s to diagnose Acute Kidney Injury at an early stage to initiate Renal Replacement Therapy as early as possible in potential patients to decrease mortality.

Department of Cardiac Anaesthesiology, Goa Medical College, Bambolim, Goa 403202

[1]MBBS, MD (Anaesthesiology), PDCC (Cardiac Anesthesia), Professor

[2]MBBS, MD (Anaesthesiology), DM (Cardiac Anaesthesiology), Associate Professor

[3]MBBS, MS (General Surgery), MCh (Cardiovascular and Thoracic Surgery), DNB (Cardiovascular and Thoracic Surgery), Professor and Head, Department of Cardiovascular and Thoracic Surgery

[4]DA, DNB, DM (Cardiac Anaesthesiology), Assistant Professor, and Corresponding Author

[5]MBBS, DPH, Assistant Lecturer, Department of Community Medicine

[6]MBBS, MD, PSM, Professor and Head, Department of Community Medicine

[7]MBBS, MS (General Surgery), MCh (Cardiovascular and Thoracic Surgery), Associate Professor, Department of Cardiovascular and Thoracic Surgery

[8]MBBS, Assistant Lecturer, Department of Cardiovascular and Thoracic Surgery

Received on : 13/10/2021
Accepted on : 22/04/2022

increased ICU stay, increased duration of ventilation and the need for RRT leads to a huge or substantial impact on both monetary as well as human resources.

Pathophysiology of Cardiac Surgery associated AKI is multifactorial and complex. Some of the risk factors for AKI that have been well documented by various studies which includes Pre-operative factors (Age, Sex, Hypertension, Diabetes, Type of Cardiac Surgery etc,) Intra-operative factors [Cardio Pulmonary Bypass (CPB) time, Aortic cross clamp time, use of blood products etc,] and Postoperative factors (use vasopressors, hypovolemia etc). To estimate the incidence of AKI among the Post Cardiac Surgery patients; to identify the risk factors associated and also to study the impact of AKI on duration of ventilation, duration of ICU, duration of hospital stay and mortality; we planned to conduct this study using Kidney Disease Improving Global Outcomes (KDIGO) guidelines in our institute and thus get some insights for the prevention of AKI and thereby decreasing the morbidity and mortality.

As per KDIGO guidelines AKI is defined as any of the following[2]

Increase in Serum Creatinine (sCr) ≥0.3 mg/dl (≥26.5 µmol/l) within 48 hours; or

- Increase in sCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or
- Urine volume of 0.5 ml/kg/h for 6 hours

Materials and Methods

The present retrospective record based observational study was conducted at Goa Medical College and Hospital Bambolim. Approval from the Institutional Ethical Committee was duly obtained prior to the conduct of the study. The data of 419 patients admitted in the ICU and CVTS wards, who underwent cardiac surgery from 1st January, 2019, till 31st December, 2019 was obtained from MRD Department.

The inclusion and exclusion criteria were as follows:

Inclusion criteria : Included all the adult patients ≥15 years who underwent Cardiac Surgery on elective/ emergency/ urgent basis in the Department of CVTS.

Exclusion Criteria : Included patients who were previously diagnosed with CKD, patients who died within 24 hours of surgery and the patients whose data was incomplete.

Depending on presence or absence of AKI, patients were divided into two groups. In our study we used KIDIGO criteria to define AKI ie, sCr increase of 0.3mg/ dl within 48 hours or an increase >50% within the previous 7 days or urine output < 0.5ml/kg/hour for 6 hours.

The following variables were studied for the present study:

- In the Pre-operative period variables analyzed included physical characteristics (Gender, Age), Biochemical parameters (baseline Serum Creatinine), and Comorbidities (Diabetes, Hypertension, COPD, Stroke), recent MI (<21 days), h/o previous cardiac Surgery, presence of Cardiac dysfunction (LVEF <40%), type of surgery performed (CABG, Valve Replacement Surgery, or the combination of the two, congenital corrections), use of Intra-aortic Balloon Pump (IABP) insertion.
- In the intra-operative period, we evaluated the duration of CPB, Aortic Cross Clamp Time, MAP on CPB, and the use of vasopressor drugs and Use of IABP.
- The variables studied in the postoperative period included MAP (first 3 post op days), duration of ventilation in hours, the duration of ICU stay in days, duration of hospital stay, use of vasopressor drugs, the initiation of RRT and the use of IABP. The Serum Creatinine values were monitored till the 7th postoperative day. Finally, the patient outcome including mortality if any was noted.

As per our institution protocol Epinephrine was used as primary vasopressor and secondary vasopressors used included Dobutamine or Milrinone or Levosimendan.

The Data was entered in Microsoft Excel and analyzed using the Statistical software namely SPSS 22.0, and R environment ver 3.2.2. Descriptive and inferential statistical analysis was carried out. Continuous variables were expressed as the Means ± Standard Deviations (SDs) and were compared with Student's t test. Categorical variables were described as frequencies and proportions and were compared with Fisher's exact tests or chi square tests. Significance is assessed at 5 % level of significance.

Results

Out of 419 patients' records reviewed it was observed that the mean age was 58.11 ±11.02 years and majority were males (69.7%). It was observed that 169 (40.3 %) out of 419 patients who underwent Cardiac Surgeries during the study period developed AKI.

Fig 1 shows 132 (31.5%) presented with stage 1 AKI whereas 24 (5.7%) and 13 (3.1%) presented with stage 2 and stage 3 respectively. The mean Serum Creatinine levels were 0.92±0.20 mg/l. Amongst the comorbid conditions studied majority had h/o Diabetes Mellitus followed by Hypertension, COPD and Stroke as shown in Table 1. Only 13(3.1%) had history of

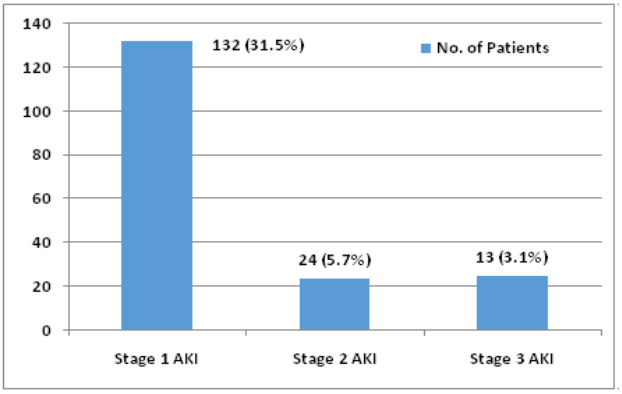

Fig 1 — Stages of AKI in patients studied

Table 1 — *KDIGO staging of AKI*

Staging of AKI		
Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline OR ≥0.3 mg/dl (≥26.5 µmol/l) increase	< 0.5 ml/kg/h for 6–12 hours
2	2.0–2.9 times baseline	<0.5 ml/kg/h for ≥12 hours
3	3.0 times baseline OR Increase in Serum Creatinine to ≥4.0 mg/dl (≥353.6 µmol/l) OR Initiation of renal replacement therapy OR, In patients <18 years, decrease in eGFR to <35 ml/min per 1.73 m^2	< 0.3 ml/kg/h for ≥24 hours OR Anuria for ≥12 hours

recent Myocardial Infarction (MI) <21 days and 8 (1.9%) had h/o previous Cardiac Surgery. The most common procedure performed was CABG followed by Valve Surgery and combination of CABG + Valve surgery.

Table 2-4 shows the association of Preoperative, intra-operative and postoperative variables with the incidence of AKI in the study subjects.

- Chi square test and Student 't' test was used as to study statistical difference between two proportion and means respectively.

Under the Pre-operative variables studied Age, sex, h/o previous Cardiac Surgery had statistically significant association with the occurrence of AKI in patients undergoing cardiac surgery. The incidence of AKI was significantly low in patients with Diabetes Mellitus (P<0.046).

Although other Surgeries like the Bentall procedures are high risk Surgeries and prone for AKI, we could not comment on the statistical association with AKI because number of cases low.

- Student 't' test was used to study significant difference between two means

Amongst the intra-operative variables it was observed that CPB duration, Aortic cross clamp time was found statistically significant. There was no significant difference between MAP in both the groups. The use of vasopressor during intra-operative period showed increased incidence of AKI, however transfusion of blood products did not show any statistical association with AKI incidence (P=0.243).

Difference in mean and proportion was calculated using student t test and chi square respectively.

As per the institution protocol Diuretics were administered for patients during postoperative period if urine output was found to be <0.5ml /kg for more than 3 hours and hence we could not assess the association between urine output and development of AKI in patients undergoing Cardiac Surgery.

In the Postoperative period MAP was monitored till the patient was in ICU. MAP was analysed for first three post op days and it was observed to have significant association with occurrence of AKI till the second postoperative day as seen in Table 4. The transfusion of blood products namely Packed cells, FFP, Platelets and the mean duration of ventilation, duration of ICU stay also had significant association (P<0.001).It was also observed that incidence of AKI significantly increased in Postoperative period with addition of vasopressors (P<0.001).

The overall mortality was 4.3% in our study with the mortality in AKI group accounting for 8.3% (Stage 1, 2, 3 accounting for 2.3%, 8.3%, 69% respectively) which was significantly higher as compared to non-AKI group ie, 1.6% as seen in Table 4.

Out of 169 patients developing AKI postsurgery 13 patients had stage 3 AKI; among which RRT was initiated in 11 patients, whereas in the non-AKI group we initiated RRT in 1 patient who underwent VSR repair and was on inotropic support. As the hemodynamic were unstable and decreasing trend of urine output in the Postoperative period we considered to initiate RRT early.

It was observed that there was no significant difference between the baseline sCr levels in both groups (P=0.868). However, there was steady rise in levels of sCr from 2nd postoperative day and levels gradually returned to baseline by day 7 as seen in Fig 2.

Usage of IABP had significant association (P<0.002) with occurrence of AKI as seen in Table 2.

Discussion

In the present study, the mean age of the patients undergoing Cardiac Surgery was 58.11 ±11.02 years which was similar to studies by TF Silva, *et al*[3], Elghoneimy, *et al*[4]. We found male predominance in

Table 2 — *Association of preoperative variables with incidence of AKI in patients studied*

Variables	AKI		Total	P Value
	No	Yes		
BASELINE CHARACTERISTICS				
Age in years :				
<40	19(4.5%)	7(1.7%)	26(6.2%)	0.043
40-60	134(32%)	77(18.4)	211(50.4%)	
>60	97(23.1%)	85(20.3%)	182(43.4%)	
Sex :				
Female	87(20.8%)	40(9.5%)	127(30.3%)	0.015
Male	163(38.9)	129(30.8%)	292(69.7%)	
COMORBID CONDITIONS				
Diabetes Mellitus :				
No	91(21.7%)	78(18.6%)	169(40.3%)	0.046
Yes	159(38%)	91(21.7%)	250(59.7%)	
Hypertension :				
No	109(26%)	71(17%)	180(43%)	0.747
Yes	141(33.6%)	98(23.4%)	239(57%)	
COPD :				
No	240(57.2)	162(38.7%)	402(95.9%)	0.942
Yes	10(2.4%)	7(1.7%)	17(4.1%)	
Stroke :				
No	244(58.2%)	162(38.7%)	406(96.9%)	0.313
Yes	6(1.4%)	7(1.7%)	13(3.1%)	
OTHER VARIABLES				
Recent MI < 21 days :				
No	240(57.3%)	166(39.6%)	406(96.9%)	0.198
Yes	10(2.4%)	3(0.7%)	13(3.1%)	
Previous Cardiac Surgeries :				
No	249(59.4%)	162(38.7%)	411(98.1%)	0.006
Yes	1(0.2%)	7(1.7%)	8(1.9%)	
Cardiac Dysfunction (LVEF) :				
< 40%	22 (5.2%)	25 (6%)	47 (11.2%)	0.056
>40%	228 (54.4%)	144 (34.4%)	372 (88.8%)	
Type of Surgery Done :				
CABG	200(47.7%)	122(29.1%)	322(76.8%)	0.063
Valve Surgery	30(7.2%)	32(7.6%)	62(14.8%)	0.050
CABG+Valve	6(1.4%)	6(1.4%)	12(2.8%)	0.489
ICR	9(2.1%)	0(0%)	9(2.1%)	0.013
Bentall procedure	0(0%)	3(0.7%)	3(0.7%)	0.034
VSR Repairs	2(0.5%)	1(0.2%)	3(0.7%)	0.804
Myxoma excision	2(0.5%)	0(0%)	2(0.5%)	0.244
Redo surgery	1(0.2%)	5(1.2%)	6(1.4%)	0.031
Total	250(59.6%)	169(40.3%)	419(99.9%)	
CLINICAL VARIABLES				
Baseline Serum Creatinine (mg/l)	0.92±0.20	0.92±0.21	0.92±0.20	0.868
IABP insertion :				
Not inserted	243(58%)	150(35.8%)	393(93.8%)	0.002
Preoperative stage	3(0.7%)	2(0.5%)	5(1.2%)	
Intraoperative stage	2(0.5%)	9(2.1%)	11(2.6%)	
Postoperative stage	2(0.5%)	8(1.9%)	10(2.4%)	
Total	250(59.7%)	169(40.3%)	419(100%)	

our study which was comparable to the study conducted by Machado MN, *et al*[5] ie, 52 %; whereas Wittlinger, *et al*[6] reported significantly more females developed AKI.

The incidence of AKI after Cardiac Surgery was 40.3% which was similar to the findings by Ramos KA, *et al*[7] ie, 43.66%, however studies conducted by Gangadharan, *et al*[8]and TF Silva[3] it was reported as 9.25% and 83.8% respectively. The variation in incidence is probably due to different criteria used to define AKI.

We found Diabetes was the most common comorbid condition followed by Hypertension, COPD and the predominant procedure performed was CABG followed by Valve Surgery and combination of both which was similar to various other studies (Machado MN, *et al*,TF Silva, *et al*)[5,3].

Redo Surgeries also carried a significant association with AKI, this might be due to the high requirement of blood products and vasopressors.

In our Study Age, Sex, h/o previous Cardiac Surgery had statistically significant association with the occurrence of AKI in patients undergoing Cardiac Surgery (P<0.005), which was contrasting to the findings by Freeland K, *et al*[9] where Age, Gender, Comorbid condition were not predictors of AKI (p<0.005). A study by Xiangcheng Xie, *et al*[10]showed significant association between Coronary Artery Disease and Hypertension (P<0.001).

We did not find any significant association between MI<21 days(p <0.65) and AKI, however S Hanuora, *et al*[11] found those with h/o of MI<30 days were more liable for development of AKI.

The intra-operative period MAP on CPB in mmHg did not show any difference between two groups in our study which was similar to the finding by Freeland K, *et al*[9]. However CPB duration, Aortic Cross Clamp Time was significantly associated with the incidence of AKI which was similar to S. Hanoura, *et al*[11]and Xiangcheng Xie, *et al*[10].Results demonstrated by Xiangcheng Xie, *et al*[10] showed CPB time longer than 110 minutes was an independent risk factor for AKI.

During the Postoperative period, the AKI group had significantly lower MAP on day 1 and 2 and explains the use ofmore vasopressors compared to non-AKI patients which shows that the higher MAP is required to prevent AKI during the early Postoperative period.

Insertion of IABP had significant association with AKI irrespective of the timing of insertion which was

Table 3 — *Association of Intra-operative variables with incidence of AKI in patients studied*

Variables	AKI		Total	P Value
	No	Yes		
CPB duration (mins)	138.79±39.59	150.63±54.64	143.56±46.56	0.010
Aortic Cross Clamp Time (mins)	92.99±28.88	101.02±40.45	96.23±34.21	0.018
MAP on CPB (mmHg)	56.62±4.56	56.81±6.29	56.69±5.32	0.720
Vasopressors	1.34±1.39	2.15±1.99	1.66±1.70	<0.001
Transfusion Of Blood Products	0.31±0.52	0.25±0.45	0.28±0.49	0.243

Table 4 — *Association of Postoperative variables with incidence AKI in patients studied*

Variables	AKI		Total	P Value
	No	Yes		
Mean Arterial Pressure (MAP) :				
MAP Day 1 (mmHg)	72.62±9.88	68.62±10.13	71.01±10.16	<0.001
MAP Day 2 (mmHg)	84.15±10.08	80.59±11.92	82.72±10.99	<0.001
MAP Day 3 (mmHg)	87.17±9.72	86.30±12.31	86.82±10.84	0.421
Transfusion of Blood Products :				
Packed cells	1.19±1.40	1.82±2.15	1.44±1.77	≤0.001
FFP	0.18±0.59	0.54±1.05	0.33±0.83	≤0.001
Platelets	0.54±1.88	1.50±3.47	0.93±2.68	≤0.001
Vasopressors Added :				
Nil	222(88.8%)	120(71%)	342(81.6%)	<0.001
One	3(1.2%)	6(3.6%)	8(1.9%)	
2 or more	25(10%)	43(25.4%)	68(16.2%)	
Other Variables :				
Duration of Ventilation (hrs.)	23.87±23.09	38.48±62.27	29.74±43.85	<0.001
Duration of ICU Stay (days)	5.02±1.85	6.12±3.15	5.46±2.51	<0.001
Duration of Hospital stay (days)	8.90±7.85	9.95±4.82	9.32±6.81	0.123
Outcome :				
Mortality	4(1.6%)	14(8.3%)	18(4.3%)	<0.001
Discharged	246(98.4%)	155(91.7%)	401(95.7%)	

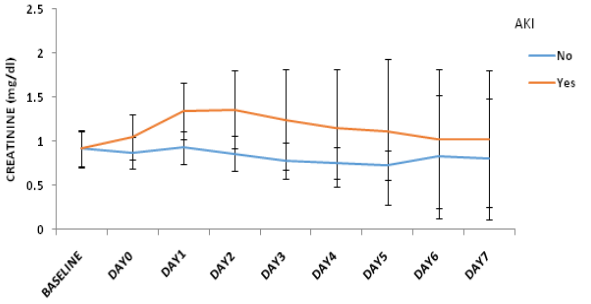

Fig 2 — Association of Serum Creatinine with incidence of AKI in patients studied

similar to finding by Wittilinger, *et al* who found that IABP is an independent risk factor for development of AKI.

We found that duration of ventilation and ICU stay (P<0.001) also showed significant association, which was similar to findings by Yanli liu, *et al*[12]. Study by Xiangcheng, *et al*[10]showed mechanical ventilation duration greater than 9 hours was an independent risk factor in the development of AKI. However some studies showed null association presumably due to limited sample size and heterogeneity of the patients (Boyles, *et al*[13]).

In our study the mortality in AKI group was 8.3% compared to non AKI group (1.6%), however in a study conducted by HY Fu, *et al*[14]AKI mortality was reported as 30.4 % mortality in comparison to non AKI ie, 8% (P<0.001).

High mortality was seen in patients initiated on RRT, which may be related to the timing of the initiation of RRT. The patient who was initiated early had a good outcome. This gives an impetus for us to search for those biomarkers which determines AKI early and probably also indicate the timing of RRT initiation. In a study by Yanli liu, *et al*[12] stage 3 AKI patients needed RRT.

Conclusion

From our study findings we conclude that AKI in patients undergoing Cardiac Surgery has significant impact on the outcome of the patients in terms of mean duration of ventilation, duration of ICU stay and also mortality; hence prevention of modifiable risk factors and early diagnosis is the key to decrease the mortality and morbidity associated with AKI. Duration of CPB and aortic cross clamp time was found to be a significant risk factor for AKI which needs to be investigated further to reduce incidence of AKI. The other modifiable risk factor includes the usage of blood products can be reduced by using methods like hemofiltration and cell savers and also achieving a proper Haemostasis. Also, there is need to find out biomarker/s to diagnose AKI at an early stage to initiate RRT as early as possible in potential patients to decrease mortality. We look forward to strategies to identify those at risk of AKI Post Cardiac Surgery and develop approaches to improve the outcome.

References

1 Makris K, Spanou L — Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. *Clin Biochem Rev* 2016; **37(2):** 85-98.

2 Khwaja A — KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012; **120(4):** c179-84.

3 TF Silva TF, Silva KR, Nepomuceno CM — Incidence of acute kidney injury post cardiac surgery/ : a comparison of the

AKIN and KDIGO criteria. *Brazilian Journal of Anesthesiology* 2021; **71(5):** 511-6.
4 Elghoneimy YA, Al Qahtani A, Almontasheri SA — Renal Impairment After Cardiac Surgery: Risk Factors, Outcome and Cost Effectiveness. *Cureus* 2020; **12(11):** e11694.
5 Machado MN, Nakazone MA, Maia LN — Acute kidney injury based on KDIGO (Kidney Disease Improving Global Outcomes) criteria in patients with elevated baseline serum creatinine undergoing cardiac surgery. *Rev Bras Cir Cardiovasc* 2014; **29(3):** 299-307.
6 Wittlinger T, Maus M, Kutschka I — Risk assessment of acute kidney injury following cardiopulmonary bypass. *J Cardiothorac Surg* 2021; **16(4):** 1-7 .
7 Ramos KA, Dias CB — Acute Kidney Injury after Cardiac Surgery in Patients Without Chronic Kidney Disease. *Braz J Cardiovasc Surg* 2018; **33(5):** 454-61.
8 Gangadharan S, Sundaram KR, Vasudevan S, Ananthakrishnan B, Balachandran R, Cherian A, *et al*— Predictors of acute kidney injury in patients undergoing adult cardiac surgery. *Ann Card Anaesth* 2018; **21(4):** 448-54.
9 Freeland K, Hamidian Jahromi A, Duvall LM, Mancini MC — Postoperative blood transfusion is an independent predictor of acute kidney injury in cardiac surgery patients. *J Nephropathol* 2015; **4(4):** 121-6.
10 Xie X, Wan X, Ji X —. Reassessment of Acute Kidney Injury after Cardiac Surgery: A Retrospective Study. *Intern Med* 2017; **56(3):** 275-282.
11 Hanoura S, Omar AS, Osman H, Sudarsanan S, Eissa M, Maksoud M, Khulaifi AA — Prevalence and predictors of acute kidney injury after cardiac surgery: a single-centre retrospective study in Qatar. *Netherlands Journal of Critical Care* 2018; **26(1):** 14-9.
12 Liu Y, Shang Y, Long D, Yu L — Intraoperative blood transfusion volume is an independent risk factor for postoperative acute kidney injury in type A acute aortic dissection. *BMC Cardiovasc Disord* 2020; **20(1):** 446.
13 Boyle JM, Moualla S, Arrigain S, Worley S, Bakri MH, Starling RC, *et al* — Risks and outcomes of acute kidney injury requiring dialysis after cardiac transplantation. *Am J Kidney Dis* 2006; **48(5):** 787-96.
14 Fu HY, Chou NK, Chen YS, Yu HY — Risk factor for acute kidney injury in patients with chronic kidney disease receiving valve surgery with cardiopulmonary bypass. *Asian J Surg* 2021; **44(1):** 229-34.