Chemistry Research Journal, **2018, 3(5):73-80**

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

The Effects of Aortic Cross-Clamp and Pump Times on Oxidative Damage and Antioxidant Parameters in the Patients Undergoing Open-Heart Surgery

Ayşe Doğan[1]*, Fevzi Sarper Türker[2], Mine Erişir[3], Gonca Ozan[3], Yusuf Özşensoy[4]

[1]Bitlis Eren University Health High School Department of Physiotherapy and Rehabilitation, 13000, Bitlis, Turkey
[2]University of Health Sciences Elazığ Training and Research Hospital Cardiovascular Surgery, 23000, Elazığ, Turkey
[3]Fırat University Faculty of Veterinary Science Department of Biochemistry, 23000, Elazığ, Turkey
[4]Cumhuriyet University, Faculty of Veterinary Medicine, Department of Veterinary Biometrics and Genetics, 58140, Sivas, Turkey

Abstract *Background:* The aim of the present study is to determine the correlation between the oxidative stress marker and the antioxidant parameters with the aortic cross clamp and pump times and to make contribution to the related studies.

Methods: Fifty four elective patients for whom cardiopulmonary bypass operation was planned with different indications were included in this study. While sixteen of fifty four patients were female, thirty eight patients were male. MDA, GPx, GSH, CAT, and SOD were investigated in the blood samples.

Results: When the change in oxidative damage and antioxidant parameters in preoperative, operative, and postoperative periods was examined in the present study; a statistically significant increase was observed in GSH level and CAT enzyme activity in operative and postoperative periods compared to preoperative period. In the present study, it was observed that there was no statistical difference in preoperative, operative, and postoperative periods in terms of cross-clamp period and pump times.

Conclusion: There was no sufficient data regarding the efficiency of aortic cross-clamp time and especially the pump time in this oxidative damage. It is recommended to conduct comprehensive studies in order to obtain sufficient, precise and reliable data regarding its effect in the oxidative damage. Additionally, the fact that CPB triggers oxidative damage may be reduced or prevented by the studies to be conducted on antioxidant treatment in CPB with same sensitivity. Thus, inhibition of different disease complications may allow patients undergoing necessarily a CPB operation to maintain a more qualified life.

Keywords Cross-clamp time, pump time, MDA, antioxidants

Introduction

Cardiopulmonary bypass (CPB) is a part of the cardiac operation that causes damage in many organs [1]. These effects occur due to a systemic inflammatory response to CPB and the effect of ischemia-reperfusion on other organs such as myocardium and lungs [2-7]. Radical oxygen species (ROS) production is an important factor in CPB-related myocardial dysfunctions [8]. Main sources of ROS are mitochondrial cytochrome oxidase, arachidonic acid pathway, xanthine oxidoreductase system and NADPH oxidase system in neutrophil granulocytes [1].

Excessive formation of ROS causes post-oxidative cellular modification by reacting with many molecules (glutathione, DNA, proteins, membrane phospholipids) and is effective in the pathogenesis of post-ischemic contractile dysfunction and dysrhythmias. ROS such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and peroxynitrite ($ONOO^-$) causes oxidative stress in the heart [9]. Oxidative reactions can increase complications after or during coronary artery bypass grafting (CABG) operation [10]. Aortic cross clamping leads to ischemia-reperfusion by increasing the oxidative stress during the operation [11]. In surgical intervention; CPB, ischemia, and reperfusion can cause myocardial stress [12]. Also in a study conducted in 2015, long-term anesthesia, CPB, and postoperative intubation periods caused prolonged intensive care period after CABG and the need of the patients for positive inotrope agents through blood transfusion [13]. In addition to many factors, prolonged surgery, CPB periods, and postoperative complications are the risk factors in gastrointestinal system complications developing after the cardiac surgery [14].

Antioxidant molecules can lessen the effect of ROS by directly reacting with reactive radicals [9]. Antioxidants such as propofol, L-arginine and N-acetylcysteine can be administered by intravenous infusion or cardioplegia in order to eliminate the effect of ROS in CPB. Also, mini-CPB method can reduce the oxidative stress markers compared to the traditional CPB method [15].

The aim of the present study is to determine the correlation between the oxidative stress marker and the antioxidant parameters with the aortic cross clamp and pump times and to make contribution to the related studies.

Material and Methods

Population of the Study

In this study, fifty four elective patients, for whom cardiopulmonary bypass operation was planned with different indications, were included. While sixteen of fifty four patients were female, thirty eight patients were male. All the patients were hospitalized in the service one or two day(s) before, they were daily hydrated with 1000 cc physiological saline solution as a routine and N-acetylcysteine 2x300 mg was administered intravenously (iv). After preoperative preparations of the patients were completed, they underwent the operation.

While the age range of the patients undergoing the operation varied between 20 and 77, their average age was 61 years. Ross procedure was applied to a twenty-year-old female patient.

Mitral valve replacement was conducted for nine patients, mitral ring and coronary revascularization were applied to one patient, aortic and mitral valve replacement were conducted for one patient, and coronary revascularization and aortic valve replacement were conducted for one patient. 41 patients underwent only coronary bypass surgery and average graft number of 43 patients undergoing coronary revascularization was 3.37. There was no postoperative early mortality.

All the patients undergoing elective cardiopulmonary bypass were included in this study. They were informed about the study and they signed the informed consent form stating that they were voluntary to participate in the study. The protocol of the present study was reviewed and approved by the ethics committee of Fırat University (Reg. No. 2015015).

In the present study, blood was drawn from each patient 3 times: before the surgical intervention (preoperative period), at the end of the surgical intervention (peroperative period) and approximately 24 hours after the surgical intervention (postoperative period). In these blood samples, the biochemical parameters were examined. These parameters were malondialdehyde (MDA), an oxidative damage indicator, and reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) which are the antioxidant indicators.

Surgical Technique

All patients were managed by the same surgical and anesthetic team in the same operation room. Six-channel electrocardiogram (ECG) and non-invasive arterial pressure monitoring was applied to the patient onto the operating table. Before the anesthesia induction, radial artery catheter was inserted under local anesthesia and preoperative blood samples were taken together with the baseline blood gas values and the invasive pressure monitoring was performed. Anesthesia induction was carried out by using 100 mg lidocaine (iv), 300 mg magnesium iv., 100 µgr fentanyl iv., 0.60-1.2 mg/kg esmeron iv., and 2 mg/kg propofol iv.. Central venous cannula and urine catheters were inserted in the post-anesthesia period. Maintenance of general anesthesia was performed by adding 20 mg esmeron and 100 µgr fentanyl (iv) in the oxygenator reservoir every 30 minutes.

While propofol infusion (%1) was performed as 20 ml/hour iv infusion dose before CPB, it was reduced to 10 ml/hour iv infusion dose during CPB. A median sternotomy was performed to all the patients. After 350-400 U/kg heparin was administered to left internal mammary artery (LIMA) prior to cannulation, which was followed by routine aortic and right atrial cannulation. For mitral valve surgery and Ross procedure, bicaval cannulation was performed. Membrane oxygenators and moderate systemic hypothermia were used to carry out the cardiopulmonary bypass (CPB). Myocardial protection was achieved by using antegrade mild hypothermic blood cardioplegia (32˚C) and repeated every 20 minutes for valve operations. Retrograde cardioplegia cannula was used for myocardial protection and cardiac deairing. Cold blood cardioplegia was prepared by adding 2 mmol/L magnesium sulphate, 5 mmol/L potassium chloride, and 1.6 gr/1000 cc sodium bicarbonate in every 1000 cc blood taken from the reservoir. Activated clotting time was maintained for >400 sec during the procedure. Mean blood pressure was kept at 60 mmHg and over. All the proximal saphenous vein anastomosis was performed via cross clamp by using a single clamp technique. Air was discharged from the proximal anastomoses for valve operation in aortotomy or left atriotomy and cross clamp was removed. After sufficient cardiac performance was provided, pump flow was reduced and CPB was ended. Heparin was neutralized with protamine at the ratio of 1:1.3 for 10 minutes after CPB.

After the operation, all of the patients were followed up in the intensive care unit**.** Second (peroperative) blood samples were taken from the radial artery catheter together with the blood gas immediately after the patient arrived to the intensive care unit. Third (postoperative) blood samples were taken approximately 24 hours after the operation. During this period the intensive care follow-up of the patient continued. All the samples were sent to the laboratory as soon as possible and they were appropriately prepared and stored.

Sample Collection

After blood samples were taken in two heparin-containing experimental tubes by using the cannula inserted in radial artery, they were taken to the laboratory for invasive blood pressure monitoring**.** While one of the heparinized blood samples was used as the whole blood, the other heparinized blood sample was centrifuged at 3000 rpm for 5 minutes and its plasma was separated and then washed three times by using physiological saline solution. Then, it was kept at the deep-freezer at -80ºC before biochemical analyses.

Biochemical Analyses

Lipid Peroxidation

The MDA assay in plasma was carried out with slight modifications based on the method of Placer et al. [16]. The MDA formed a pink complex with thiobarbituric acid (TBA) and the absorbance read was 532 nm. The plasma MDA content was expressed as nmol/ml.

GSH Level

Being determined in accordance with the method of Chavan et al. [17] the GSH contents were expressed as µmol/ g Hb.

CAT Activity

The Aebi [18] method was used to measure CAT activity. The rate of degradation of H_2O_2 by CAT was spectrophotometrically measured by using H_2O_2 absorption light at 240 nm wavelength. CAT activity was calculated as katal/g Hb.

SOD Activity

The SOD enzyme activity was measured based on degradation of nitroblue tetrazolium (NBT) by the superoxide radical, which was produced by the xanthine- xanthine oxidase system. The blue-colored formazan obtained at the end of the reactions was maximally absorbed at 560 nm [19]. The SOD enzyme activity was calculated as U/g Hb.

Statistical Analysis

Statistical analysis was carried out using the SPSS package program (15.0 for Windows). T-test was used to assess data of cross-clamp time and pump time in themselves in terms of oxidative damage and antioxidant parameters. Paired t-test was used to compare the on-pump data in terms of oxidative damage and antioxidant parameters. Also, the Mann-Whitney U test was used to evaluate the routine biochemistry data of groups in themselves. The value of p <0.05 was accepted as statistically significant. All of the results were shown as mean ± standard error mean (SEM) for parametric tests and median for non-parametric tests.

Results and Discussion

In the present study, the change in oxidative damage and antioxidant parameters in preoperative, operative, and postoperative periods was examined. A statistically significant increase was determined in GSH level (P<0.001) and CAT enzyme activity (P<0.05) in the operative and postoperative periods compared to preoperative period. However, no significant difference was determined in the MDA level and SOD enzyme activity (P>0.05) (Table 1).

Table 1: Statistical comparison of oxidative and antioxidant parameters in preoperative, operative, and postoperative periods. (Mean±SEM)

Parameters	Preoperative period	Operative period	Postoperative period	p
MDA nmol/ml	3.20±0.13	3.23±0.16	3.30±0.18	P>0.05
GSH µmol/g Hb	1.66±0.11[a]	2.56±0.27[b]	3.13±0.39[b]	P<0.001
CAT k/g Hb	25.85±2.06[a]	35.66±2.58[b]	35.97±3.93[b]	P<0.05
SOD U/g Hb	34.21±1.16	36.36±1.15	36.36±1.61	P>0.05

Letters a and b show statistically different groups

When cross-clamp times were compared in the present study, it was found that there was a decrease in GSH level and an increase in other parameters in the group of cross-clamp time of > 60 min compared to the group of a cross-clamp time of <60 min, but no significance was determined between the groups in terms of all the parameters (P>0.05) (Table 2).

Table 2: The changes in the oxidative damage and antioxidant parameters in preoperative, operative and postoperative periods in the aortic cross-clamp times of less than 60 min and greater than 60 min. (Mean±SEM)

Parameters	Preoperative period	Operative period	Postoperative period
Less than 60 min MDA nmol/ml	3.05±0.23	3.26±0.27	3.30±0.30
Greater than 60 min MDA nmol/ml	3.30±0.18	3.21±0.20	3.31±0.24
Less than 60 min GSH µmol/g Hb	1.89±0.25	3.01±0.64	3.63±0.86
Greater than 60 min GSH µmol/g Hb	1.53±0.11	2.31±0.21	2.85±0.37
Less than 60 min CAT k/g Hb	22.32±3.08	30.19±2.58	28.00±4.23
Greater than 60 min CAT k/g Hb	27.96±2.70	38.92±3.73	40.71±5.64
Less than 60 min SOD U/g Hb	32.84±1.80	34.06±1.69	35.48±2.84
Greater than 60 min SOD U/g Hb	35.03±1.52	37.73±1.52	36.89±1.97

When the groups of pump time < 100 min and > 100 min were compared in terms of oxidative damage and antioxidant parameters; no statistical difference was determined between the groups in preoperative, operative, and postoperative periods (P>0.05) (Table 3).

Table 3: The changes in the oxidative damage and antioxidant parameters in preoperative, operative, and postoperative periods in the pump times of less than 100 min and greater than 100 min. (Mean±SEM)

Parameters	Preoperative period	Operative period	Postoperative period
Less than 100 min MDA nmol/ml	3.24±0.17	3.36±0.20	3.44±0.21
Greater than 100 min MDA nmol/ml	3.16±0.23	3.02±0.27	3.10±0.34
Less than 100 min GSH µmol/g Hb	1.76±0.17	2.95±0.40	3.33±0.55
Greater than 100 min GSH µmol/g Hb	1.50±0.14	1.97±0.26	2.84±0.53
Less than 100 min CAT k/g Hb	27.11±2.85	35.48±2.71	36.32±4.42
Greater than 100 min CAT k/g Hb	23.15±2.55	34.25±5.03	34.98±7.00
Less than 100 min SOD U/g Hb	34.22±1.36	36.53±1.39	38.40±2.04
Greater than 100 min SOD U/g Hb	33.40±2.04	34.56±2.15	32.76±2.26

Table 4: Demographic data of the patients

Σn	Groups	Urea	Creatinine	AST	ALT	CRP
54		(30.50 ± 2.59) (39.40 ± 2.31)	(0.68 ± 0.23) (0.92 ± 0.21)	(17.25 ± 2.01) (20.68 ± 3.59)	(17.25± 1.25) (22.18± 2.59)	(9.48 ± 2.56) (8.70 ± 1.67)
Presence of DM	Yes No	0.125	0.213	0.189	0.224	0.682
Presence of HT	Yes No	0.568	0.492	0.087	0.489	0.321
Smoking status	Smoking Not Smoking	0.705	0.867	0.092	0.152	0.457
Presence of COPD	Yes No	0.055	0.138	0.271	0.656	0.816

No statistical significance was found in the biochemistry data of the groups (P>0.05) (Table 4).

The previous studies have revealed that patients undergoing CPB can get damaged due to the increase in multiple oxidative damage markers and the decrease in the antioxidant reserves, for this reason, postoperative morbidity and hospitalization periods may prolong [20-22]. Also in a study conducted in 2017, 1960 patients undergoing the on-pump procedure were assessed and it was determined that postoperative complications, intensive care period, and mechanical ventilation period increased in the patients for whom CPB period extended 180 minutes and CPB period also had a significant effect on mortality [23]. It was determined that CPB/graft period of greater than 56 minutes had a directly determinant effect on mortality and led to length of intensive care stay of > 48 hours and a mechanical ventilation period of > 24 hours [23]. In the present study, the significant increase in GSH and CAT antioxidant parameters in the patients undergoing CPB in operative and postoperative periods compared to preoperative period may have caused the insignificant increase in MDA in operative and postoperative periods. In parallel to previous studies, high antioxidant parameters in the patients in operative and/or postoperative periods were shown to be important. Thus, postoperative morbidity associated with the oxidative damage and/or possible damages caused by different complications can be prevented in the patients undergoing CPB operation.

In a previous study, a significant difference was determined between 5-min and 60-min cross-clamp times in terms of 8- isoprostane, an oxidative damage marker, and the oxidative damage increased in these cross-clamp times [1]. In the present study, no significant difference was determined between the groups of cross-clamp times of > 60 min and < 60 min in preoperative, operative and postoperative periods in terms of MDA, an oxidative damage marker.

In a previous study, the 8-isoprostane was examined in the blood samples taken in five time periods as 0^{th} min, 5^{th} min, 1^{st} hr, 12^{th} hr, and 24^{th} hr and consequently while a significant increase was determined at the end of 5^{th} min, 1^{st} hr and 12^{th} hr compared to 0^{th} min, no significant difference was found in the samples at 24^{th} hr compared to 0^{th} min. [1]. This result shows a parallelism with the present study indicating that there was no change in the MDA level selected as the oxidative damage parameter in operative and postoperative periods than preoperative period. This result obtained as a result of the present study was thought to be associated with increases especially in GSH level and CAT activity in operative and postoperative periods compared to preoperative period. Also, it was thought that these increases in GSH level and CAT activity suppressed the increase in MDA level in operative and postoperative periods.

In another study, average aortic cross-clamp time was determined as 50 min and the 8-isoprostane levels were measured in the patients with a cross-clamp time of less than 50 min and those with a cross-clamp time of greater than 50 min. In the comparison of the 8-isoprostane in these groups, a significant difference was found in the blood samples taken at the 5^{th} min and 1^{st} hour [1]. A significant increase was observed in the 8-isoprostane levels at the 5^{th} min and 1^{st} hr in the group of cross clamp time of greater than 50 min compared to the other group; however, no difference was found between the groups having a cross clamp time of less than 50 min and greater than 50 min in terms of 8-isoprostane in the comparison of the blood samples taken at 12^{th} hr and 24^{th} hr. In other words, it was determined that the cross-clamp time had no effect on oxidative damage after the 1^{st} hr [1]. In the present study, no significant difference was determined in MDA and GSH levels and CAT and SOD enzyme activities in the groups having an average aortic cross-clamp time of less and more than 60 minutes in operative (210±28 min) and postoperative (approximately 24 hours) periods compared to preoperative period. No difference was determined between the groups with a cross-clamp time of less and more than 60 min in terms of oxidative damage in the operative (210±28 min) and postoperative (approximately 24 hours) periods compared to the preoperative period. This result shows parallelism with the results of the study by Jose Garcia-de-la Asuncion et al. [1].

In various studies on bypass operation, on-pump and off-pump bypass operations have been investigated in terms of oxidative damage and antioxidant parameters [24] however differently from these studies, in the present study, the pump time was statistically assessed in terms of oxidative damage. As a result of the statistics, it was found that there was no significant difference between the groups having an average pump time of less than 100 min and greater than 100 min in terms of MDA and GSH levels and CAT and SOD enzyme activities. It was considered that while the on-pump operation had an effect in terms of oxidative damage and antioxidant parameters compared to the off-pump operation [24], pump time had no effect on oxidative damage and antioxidant parameters. Due to a limited number of related studies, the pump time should be examined further in terms of oxidative damage.

As a result, in the present study, the oxidative damage associated with CPB was prevented by being suppressed by the antioxidant parameters especially in operative and postoperative periods. This result showed the importance of the antioxidants in the elimination or suppression of negative effects of a procedure [8] causing oxidative damage such as CPB. Further clinical related studies are important.

Aortic cross-clamping stops the blood flow in the coronary arteries and causes myocardial ischemia [25]. In the studies, oxidative damage has been determined especially in the blood samples taken at the 5^{th} min and 1^{st} hr cross-clamp times however the further results have not revealed significantly the correlation between cross-clamp time and the oxidative damage, which shows parallelism with the present study.

Insufficient data regarding the correlation between the pump time and oxidative damage and also the lack of a statistical difference in the comparison of the groups of pump time of < 100 min and > 100 min in terms of oxidative damage and antioxidant parameters in the present study have revealed that there is a need of further and detailed studies on the bypass operation especially regarding this section with insufficient data.

Conclusion

The present study and numerous studies [24] have specified the correlation of CPB with oxidative damage. However, in this oxidative damage, there was no sufficient data regarding the efficiency of aortic cross-clamp time and especially the pump time. Thus, especially these issues were emphasized in the present study. The fact that CPB triggers oxidative damage may be reduced or prevented by making comprehensive studies, obtaining sufficient, precise and reliable data regarding its effect in the oxidative damage and also conducting the studies on antioxidant treatment in CPB with same sensitivity. Thus, prevention of different disease complications may allow patients undergoing necessarily a CPB operation to maintain a more qualified life.

Conflict of Interest Statement

The authors stated that they have no conflict of interest regarding the publication of this article.

References

1. Garcia-de-la- Asuncion, J., Pastor, E., Perez-Griera, J., Belda, FJ., Moreno, T., Garcia-del-Olmo, E., Marti, F. (2013). Oxidative stress injury after on-pump cardiac surgery: Effects of aortic cross clamp time and type of surgery. *Redox Report Communications in Free Radical Research*, 18(5):194-199.
2. Becker, LB. (2004). New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardşovas Res,* 61: 461-70.
3. Clermont, G., Vergerly, C., Jazayeri, S., Lahet, JJ., Goudeau, JJ., Lecour, S., et al. (2002). Systemic free radical activation is a major event involved in myocardial oxidative stress related to cardiopulmonary bypass. *Anesthesiology,* 96: 80-7.
4. Bolli, R. (1991). Oxygen-derived free radicals and myocardial reperfusion injury an overview. *Cardivasc Drug Ther,* 262: 249-268.
5. Vaage, J., Valen, G. (1993). Physiology and mediators of ischemia-reperfusion injury with special reference to cardiac surgery. *Scand J Thor Cardiovasc Surg,* 41: 1-18.
6. Ferrari, R., Alfieri, O., Curello, S., Ceconi, C., Cargnoni, A., Marzollo, P., et al. (1990). Occurrence of oxidative stress during reperfusion of the human heart. *Circulation,* 81: 201-11.
7. Ng, CS., Wan, S., Yim, AP., Arifi, AA. (2002). Pulmonary dysfunction after cardiac surgery. *Chest*, 121: 1269-77.
8. Kevin, LG., Novalija, E., Stowe, DF. (2005). Reactive oxygen species as mediators of cardiac injury and protection: the relevance to anesthesia practice. *Anesth Analg,* 1275-87.
9. Zakkar, M., Guida, G., Suleiman, MS., et al. (2015). Cardiopulmonary bypass and oxidative stress. *Hindawi Publising Corporation Oxidative Medicine and Cellular Longevity.*
10. Cavalca. V, Sisillo. E, Veglia. F, et al. (2006). Isoprostanes and oxidative stress in off-pump and on-pump coronary bypass surgery. *Annals of Thoracic Surgery,* 81(2): 562-567.
11. Oktay, V., Baydar. O., Sinan, UY., et al. (2014). The effect of oxidative stress related with ischemia-reperfusion damage on the pathogenesis of atrial fibrillation developing after coronary artery bypass graft surgery. *Türk Kardiyol Dern, Arş (Arch Turk Soc Cardiol),* 42(5): 419-425.
12. Türker, FS., Doğan, A., Ozan, G., Kurtuluş, K., Erışır, M. (2016). Change in Free Radical and Antioxidant Enzyme Levels in the Patients Undergoing Open Heart Surgery with Cardiopulmonary Bypass. Hindawi Publising Corporation Oxidative Medicine and Cellular Longevity.
13. Azarfin, R., Ashouri, N., Totonchi, Z., Bakhshandeh, H., Yaqhoubi, A. (2014 Oct 14). Factors influencing prolonged ICU stay after open heart surgery. *Res Cardiovasc Med,* 3(4): e20159.
14. Allen, SJ. (2014 June). Gastrointestinal complications and cardiac surgery. *J Extra Corpor Technol,* 46(2): 142-9.
15. Gerritsen, WB., van Boven, WJP., Boss, DS., et al. (2006). Malondialdehyde in plasma, a biomarker of global oxidative stress during mini-CABG compared to on- and off-pump CABG surgery: a pilot study. *Interactive Cardiovascular and Thoracic Surgery,* 5(1): 27-31.

16. Placer, ZA., Cushman, L., Johnson, BC. (1966). Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. *Anal Biochem*, 16: 359-364.
17. Chavan, S., Sava, L., Saxena, V., Pilliai, S., Sontakke, A., Ingole, D. (2005 Jan). Reduced glutathione: Importance of specimen collection. *Indian J Clin Biochem*, 20(1): 150-152.
18. Aebi, H. (1984). Catalase. *In vitro Methods Enzymol*, 105: 121-126.
19. Sun, Y., Oberley, WL., Li ,YA. (1988). Simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34(3): 97-500.
20. Leong, JY., van der Merwe, J., Pepe, S., Bailey, M., Perkins, A., Lymbury, R., et al. (2010). Perioperative metabolic therapy improves redox status and outcomes in cardiac surgery patients: a randomized trial. *Heart Lung Circ*, 19: 584-91.
21. Castillo, R., Rodrigo, R., Perez, F., Cereceda, M., Asenjo, R., Zamorano, J., et al. (2010). Antioxidant therapy reduces oxidative and inflammatory tissue damage in patients subjected to cardiac surgery with extracorporeal circulation. *Basic Clin Pharmacol Toxicol*, 108: 256-62.
22. Papoulidis, P., Ananiadou, O., Chalvatzoulis, E., Ampatzidou, F., Koutsogiannidis, C., Karaiskos, T., et al. (2011). The role of ascorbic acid in the prevention of atrial fibrillation after elective on-pump myocardial revascularization surgery: a single-center experience a pilot study. *Interact Cardiovasc Thorac Surg*, 12: 121-4.
23. Madhavan, S., Chan, SP., Tan, WC., Eng, J., Li, B., Luo, HD., Teoh, LK. (2017 Jul 24). Cardiopulmonary bypass time: every minute counts. *J Cardiovasc Surg (Torino)*, doi: 10.23736/S0021-9509.17.09864-0.
24. Gonenc, A., Hacışevki, A., Griffiths, HR., Torun, M., Bakkaloglu, B., Simsek, B. (2011). Free radical reaction products and antioxidant capacity in beating heart coronary artery surgery compared to conventional bypass. *Russian in Biokhimiya*, 76(6): 829-839.
25. Yildirim, F., Iskesen, I., Kurdal, AT., Ozturk, T., Taneli, F., Gozukara, C., Ozbakkaloglu, A. (2016). Is ‘’attenuation of oxidative stress’’ helpful to understand the mechanism of remote ischemic preconditioning in cardiac surgery? *Journal of Cardiothoracic and Vascular Anesthesia*, 30(1): 134-140.