Türk Göğüs Kalp Damar Cerrahisi Dergisi 2012;20(4):722-728
doi: 10.5606/tgkdc.dergisi.2012.145

***Original Article** / Özgün Makale*

Antioxidant response after the operations for congenital heart diseases

Doğuştan kalp hastalıkları ameliyatları sonrası antioksidan yanıt

Yüksel Atay,[1] Ayşenur Atay,[2] M. Fatih Ayık,[1] Figen İşleten,[2] Mehmet Köseoğlu,[2] Emrah Oğuz,[1] Çağatay Engin,[1] Tahir Yağdı[1]

[1]Department of Cardiovascular Surgery, Medical Faculty of Ege University, İzmir, Turkey;
[2]Department of Biochemistry and Clinical Biochemistry, Atatürk Education and Research Hospital, İzmir, Turkey

Background: In this study, we investigated total antioxidant response and plasma levels of albumin, total bilirubin, uric acid and high sensitive C reactive protein (hsCRP) in patients with congenital heart disease treated with surgery using on-pump and off-pump techniques.

Methods: Thirty-five patients with congenital heart diseases undergoing surgical treatment were divided into two groups: 20 patients were operated with using cardiopulmonary bypass (group 1, on-pump) and 15 patients were operated without cardiopulmonary bypass (group 2, off-pump). Blood samples were collected before surgery and at 1, 24 and 72 hours following surgery.

Results: Total antioxidant response was prompt and drew a peak within the first hour following surgery in group 1. After 24 hours, it showed a sustained increase (p=0.009). In the group 2, total antioxidant response decreased significantly within the first hour, and then increased to a peak level within the 24 hours (p=0.04). Thereafter, it was gradually reduced. Total antioxidant response, albumin, bilirubin and hsCRP levels remained high in group 1, after 24 hours. Total antioxidant response levels were positively associated with the albumin levels at 24 hours following surgery in group 2 (r=0.669, p=0.01).

Conclusion: The plasma levels of albumin may be considered in the assessment of global antioxidant response in patients treated with CPB technique.

Key words: Antioxidants; cardiac; cardiopulmonary bypass; congenital; oxidative stress; surgery.

Amaç: Bu çalışmada on-pump ve off-pump teknik ile ameliyat edilen doğuştan kalp hastalarında total antioksidan yanıt ve albümin, total bilirubin, ürik asit ve yüksek duyarlılıklı C reaktif protein (hsCRP) düzeyleri araştırıldı.

Çalışma planı: Cerrahi tedavi uygulanan 35 doğuştan kalp hastası iki gruba ayrıldı: kardiyopulmoner baypas kullanılarak ameliyat edilen 20 hasta (grup 1, on-pump) ve kardiyopulmoner baypas kullanılmadan ameliyat edilen 15 hasta (grup 2, off-pump). Kan örnekleri ameliyat öncesi ve ameliyat sonrası 1, 24. ve 72. saatlerde alındı.

Bulgular: Grup 1'de cerrahi sonrası total antioksidan yanıt ani olup ameliyat sonrası 1. saatte tepe yapmakta idi. Yirmi dördüncü saatten sonra ise, sürekli bir yükseliş gözlendi (p=0.009). Grup 2'de cerrahi sonrası 1. saatte total antioksidan yanıt düzeyi anlamlı olarak azaldı, ardından tekrar yükselerek 24. saatte tepe noktasına ulaştı (p=0.04). Daha sonra yavaş düşüş görüldü. Total antioksidan yanıt, albümin, bilirubin ve hsCRP düzeyleri grup 1'de 24. saatten sonra yüksek kalmaya devam etti. Total antioksidan yanıt düzeyi grup 2'de ameliyat sonrası 24. saatteki ölçümde albümin düzeyi ile pozitif ilişkili bulundu (r=0.669, p=0.01).

Sonuç: Plazma albümin düzeyi kardiyopulmoner bypass tekniğiyle ameliyat edilen olgularda global antioksidan yanıtın değerlendirilmesinde kullanılabilir.

Anahtar sözcükler: Antioksidanlar; kardiyak; kardiyopulmoner baypas; doğuştan; oksidatif stres; cerrahi.

Available online at
www.tgkdc.dergisi.org
doi: 10.5606/tgkdc.dergisi.2012.145
QR (Quick Response) Code

Received: December 25, 2011 *Accepted:* February 6, 2012

Correspondence: M. Fatih Ayık, M.D. Ege Üniversitesi Tıp Fakültesi Kalp ve Damar Cerrahisi Anabilim Dalı, 35100 Bornova, İzmir, Turkey.
Tel: +90 232 - 390 40 61 e-mail: fayik35@yahoo.com

The phenomena of oxidative stress (OS) is determined by the imbalance between the reactive oxygen species (ROS) and antioxidants. This is due either to an increase in the production of the former or to an inadequate defense on the part of the latter.[1] The use of cardiopulmonary bypass (CPB) and ischemic arrest in adult patients is known to mediate OS, which is an important contributor to CPB-associated postoperative complications.[2] Pediatric patients with congenital heart defects seem particularly prone to these complications,[3] and very little is known about OS in pediatric cardiac surgery either with or without CPB. Various endogenous antioxidants, such as albumin, bilirubin, and uric acid, work as a part of the antioxidant defense mechanism. Direct measurement of the ROS and antioxidant molecules is not easy because of the complexity and cost of the available techniques. The measurement of total antioxidant response (TAR) seems to be a practical way to reflect the antioxidative status of plasma because the effects of various antioxidants are additive.

The purpose of this prospective study was to analyze the operative and early postoperative changes of the TAR, high-sensitivity C-reactive protein (hs-CRP) and activated and/or generated antioxidants, including endogenous antioxidants, in infants who undergoing cardiac surgery using both on-pump and off-pump techniques.

PATIENTS AND METHODS

Patients' characteristics

We prospectively studied 35 patients undergoing elective surgery for congenital heart defects using both the on-pump and off-pump technique at the Ege University Department of Cardiovascular Surgery. We compared 20 patients (10 males, 10 females; mean age 5.2±0.9 years) who were operated on with CPB (group 1= on-pump) and 15 patients (7 males, 8 females; mean age 4.8±1.1 years) who were operated on without CPB (group 2= off-pump). The selection of either technique was made by the individual surgeon based on the type and status of heart disease and his preference. Patients who had received blood and blood products and those who had used known antioxidants such as allopurinol and captopril were excluded from the study. The patients' families were informed of the study, and the principles of Helsinki Declaration were followed. In addition, the institutional ethics committee approved the study. Patient characteristics are shown in Table 1.

Surgical technique

Standard general anesthesia was applied to all patients in the same manner, and the surgery consisted of either a median sternotomy or thoracotomy. In group 1, the CPB circuit was composed of a Sarns roller pump (Terumo Cardiovascular Systems, Ann Arbor, Michigan, USA) and a membrane oxygenator (Bentley Oxygenation System CM50, Baxter-Bentley Laboratories, Irvine, California, USA) with an incorporated cardiotomy reservoir. The pump was primed with Ringer's solution, 20% mannitol (3 ml/kg), and 8.4% sodium bicarbonate (1 mmol/kg). The flow rate was 2.4 L/min per square meter (m^2) body surface area. Heparin (Liquemine, La Roche Ltd., Basel, Switzerland) 3 mg/kg was given before cannulation. The activated clotting time (ACT) was monitored and kept at >480 seconds. Cardiopulmonary bypass was established by cannulation of the ascending aorta and the right atrium. Moderate hemodilution (down to a hematocrit of 15-34%) and moderate systemic hypothermia (a nasopharyngeal temperature of 28-30 °C) were employed. A blood cardioplegic solution was infused into the aortic root at sequences as warm induction, cold maintenance, and hot-shot, respectively. After discontinuation of CPB, the heparin was neutralized with an equipotent dose of protamine chloride (La Roche Ltd., Brussels, Belgium).

Blood sampling

The measured biochemical parameters were the plasma levels of TAR, albumin, total bilirubin, uric acid, and hs-CRP levels in both groups. Venous blood samples were collected before surgery (after an overnight fast) and at one, 24, and 72 hours afterwards. The times of the blood sampling were chosen based on both literature data and our own opinion, which both pointed to the fact that the most intense alterations occur at those times. The samples were withdrawn from a cubital vein into vacutainer tubes and immediately stored at 4 °C. The sera were then separated from the cells by centrifugation at 1500 x g for 10 minutes and stored at -20 °C until the day of analysis.

Measurement of the total antioxidant response

The TAS of the sera was measured using an automated colorimetric measurement method developed by Erel.[4]

In this method, the hydroxyl radical, which is the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate o-dianisidine. This produces the dianisidyl radical that is bright yellow-brown in color. Next, the rate of the reaction is monitored by following the absorbance of this radical. The antioxidants of the sample suppress the oxidative reactions and prevent the color change. The assay results are expressed as millimoles of trolox equivalent per liter. Within- and between-batch precisions were lower than 3%.

Table 1. Patient characteristics and operative data

	On-pump			Off-pump		
	n	%	Mean±SD	n	%	Mean±SD
Number of patients	20			15		
Age (years)			5.2±0.9			4.8±1.1
Gender						
Male	10			7		
Female	10			8		
Body surface area (m²)			0.8±0.1			0.8±0.2
Left ventricular ejection fraction			63.5±9.3			66.4±4.4
Cardiopulmonary bypass time (min)			73.6±29.1			
Cross-clamp time (min)			42.5±17.3			
Beta adrenergic receptor antagonists		23			27	
Calcium antagonists		25			20	
Diuretics		55			40	

SD: Standard deviation.

Measurement of the individual antioxidants and hs-CRP

The serum albumin, uric acid, and total bilirubin levels were measured by commercial kits using an Abbott Aeroset auto analyzer (Abbot Laboratories, Abbott Park, Illinois, USA), and the serum hs-CRP was measured by a Delta nephelometer (Radim Diagnostics, Italy).

Statistical analysis

Statistical analysis of data was performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, Illinois, USA) version 14.0 for Windows software program. Values were given as means ± standard deviation (SD) of in vitro samples that were analyzed four times. The equality of means of the independent samples were compared using Student's t-test. All p values <0.05 were considered to be significant. Relationships among variables were obtained using Pearson's correlation coefficient.

RESULTS

Perioperative data is presented in Table 2. No perioperative deaths were recorded, and no patients required exploration for postoperative bleeding. Prolonged mechanical ventilation (longer than 48 hours) was seen in four patients in group 1 and in three patients in group 2. Renal failure was seen in two patients in group 1. During the hospital stay, inotropic support (higher than 5 μg/kg/min dopamine) was needed in 35% (n=7) and 27% (n=4) of patients in groups 1 and 2, respectively. The incidence of major adverse events (death, myocardial infarction, neurological deficit) and minor adverse events (wound infection, excessive bleeding, and rhythm disturbances) was similar in both groups. Only autologous blood transfusions were applied to the patients. All were discharged within 6 to 14 days following the operation, and there was no significant difference in the duration of intensive care unit (ICU) and hospital stay between the two groups.

The preoperative levels of bilirubin and uric acid were slightly higher (p=NS) in group 2. There was a significant increase (p=0.009) in the TAR the first hour after surgery in group 1 as it was slightly decreased during the first 24 hours but increased again after that. In contrast to group 1, the TAR was significantly decreased (p=0.04) at the first hour and increased within the first 24 hours in group 2. After 24 hours, the TAR decreased in this group. The TAS was positively related to the albumin levels at 24 hours (r=0.669, p=0.01) in group 2, and the albumin, bilirubin, and hs-CRP had similar

Table 2. Operative diagnosis

	n
On-pump	
Tetrology of Fallot	7
Atrial and/or ventricular septal defect	6
Aortic stenosis	3
Single ventricle	2
Pulmonary venous return anomaly	1
Aortopulmonary window	1
Off-pump	
Tetrology of Fallot	4
Single ventricle	3
Transposition and aortic coarctation	1
Ventricular septal defect and patent ductus arteriosus	1
Aortic coarctation and subaortic stenosis	1
Middle aortic syndrome	1
Patent ductus arteriosus	2
Double outlet right ventricle and transposition	1
Pulmonary atresia and atrial septal defect	1

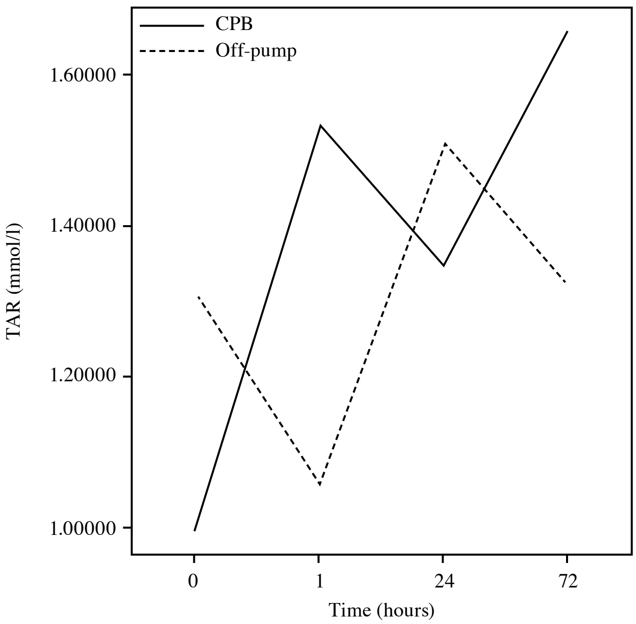

Figure 1. Changes in total antioxidant response (TAR) in patients with a congenital heart defect who had surgery with cardiopulmonary bypass (CPB) and the off-pump technique.

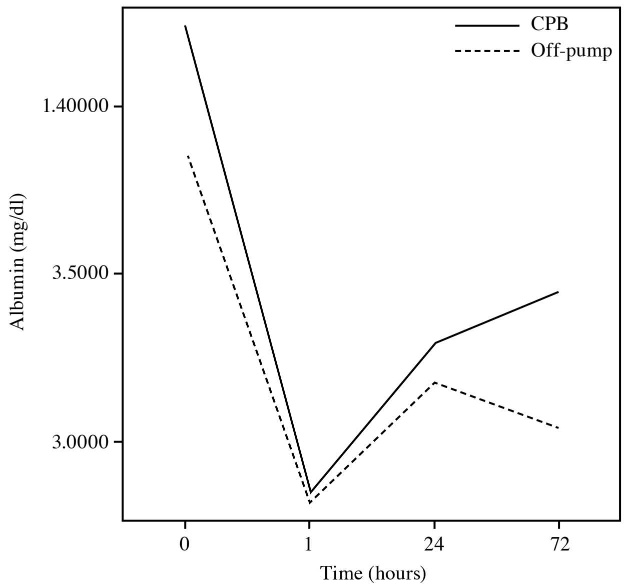

Figure 2. Changes in serum albumin in patients with a congenital heart defect who had surgery with cardiopulmonary bypass (CPB) and the off-pump technique.

curves within the first 24 hours in both groups. After that time, the TAR, albumin, and hs-CRP increased in group 1 and decreased in group 2 (Figure 1-5).

DISCUSSION

Oxidative stress, which is usually associated with the increased formation of ROS, modifies phospholipids and proteins, leading to lipid peroxidation and oxidation of thiol groups.[5,6] These lipids and thiol groups are closely linked to inflammatory responses, including complement activation, release of cytokines, and leukocyte activation along with the expression of adhesion molecules.[7] The serum concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, and costly and also require complicated techniques.[8]

Since the antioxidative effects of the antioxidant components of plasma are additive, the measurement of the TAR reflects the antioxidative status of plasma. In the present study, as previously mentioned, the total antioxidant status of the plasma was measured using an automated colorimetric measurement method developed by Erel.[4] In this method, the total antioxidant response of plasma, especially against potent free radical reactions, which strongly lead to oxidative damage of biomolecules such as lipids, proteins and DNA, is measured. In addition, hydrogen peroxide and other derivatives of peroxides, which produce physiologically and increase under some conditions, diffuse into the plasma. Here, the antioxidant components of plasma overcome them, and they are simultaneously consumed.[9] We evaluated the oxidative status of plasma by measuring the TAR.

There are only a few studies which have compared the degree of OS for patients undergoing on-pump versus off-pump techniques. In general, it has been demonstrated that the on-pump procedure gives rise

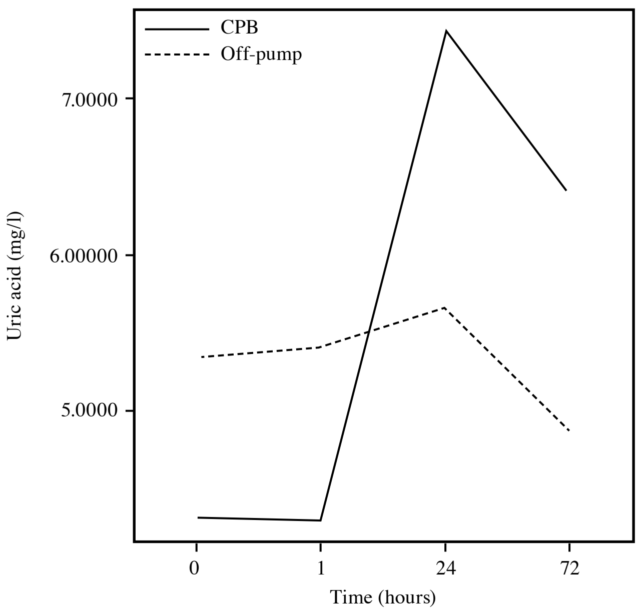

Figure 3. Changes in serum uric acid changes in patients with a congenital heart defect who had surgery with cardiopulmonary bypass (CPB) and the off-pump technique.

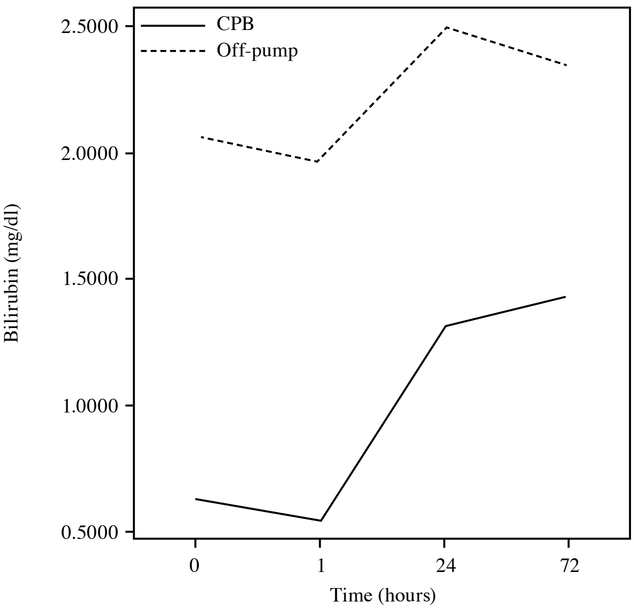

Figure 4. Changes in serum total bilirubin in patients with a congenital heart defect who had surgery with cardiopulmonary bypass (CPB) and off-pump technique.

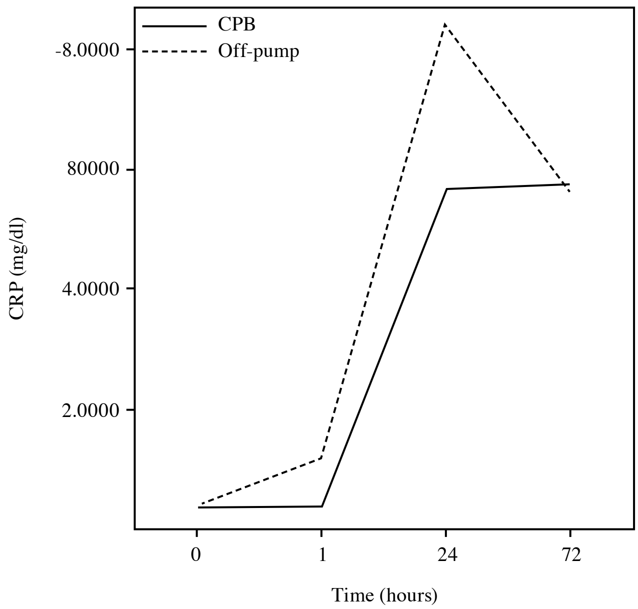

Figure 5. Changes in serum high-sensitive C-reactive protein (hs-CRP) in patients with a congenital heart defect who had surgery with cardiopulmonary bypass (CPB) and the off-pump technique.

to a more pronounced systemic inflammation and OS than the off-pump procedure.[10,11] The extracorporeal circulation apparatus, ischemia-reperfusion, and changes in body temperature are some of the sources of ROS production, and these lead to a depletion of the endogenous antioxidant response. There seem to be significant differences between the susceptibility of pediatric patients and adults to CPB surgery regarding OS. Similar to Christensen's pediatric population study,[12] Ballmer et al.[13] found that individual antioxidant levels in adult patients were significantly depleted following surgery performed with CPB. However, the antioxidant decline immediately after CPB (<20%) was much less extensive than in pediatric study patients, and the maximum decline (>50%) was not observed until 24 hours after CPB.

Oxidative stress occurs when the free radical generation exceeds the human antioxidant defense mechanisms. In the present study, both groups of patients had visible changes in antioxidant response. As metioned previously, the most intense alterations occur before surgery and at one, 24, and 72 hours after the operation. In group 1, the TAR increase was prompt and peaked within the first hour. After that, it slightly decreased during the first 24 hours. This occurrence is closely linked to CPB. After the first 24 hours, it showed a slow and sustained increase. In group 2, the TAR decreased to a minimum within the first hour and then slowly increased to a peak level within the first 24 hours, which is nearly the same as in the first hour level of group 1. Thereafter, it slowly decreased, which is in contrast to group 1. These changes indicate that severe OS in off-pump surgery is limited within the first 24 hours, but it remains constantly in on-pump surgery.

Albumin, the most abundant protein in serum, bilirubin, and uric acid work in the antioxidant defense mechanism against OS. Recent evidence indicates that albumin may provide antioxidant protection by functioning as a serum peroxidase in the presence of reduced glutathione, an intracellular antioxidant. Various epidemiological and clinical data consistently has shown that a reduced level of serum albumin is associated with an increased event and mortality risk.[14] A decreased albumin level might act as a marker for other pathogenic processes or factors, such as infection, inflammation, loss of lean mass associated with illness, undernutrition, or lack of activity, and it also reflects a serum antioxidant deficit. In our study, the albumin levels and the TAR were correlated in group 2 within the first 24 hours. In group 1, the TAR and albumin levels were not correlated during this period of time because of many factors such as hemodilution, which possibly affected the albumin level with CPB. After 24 hours, the albumin levels and the TAR had similar curves in group 1.

As early as 1959, it was suggested that bilirubin might be an antioxidant, and it can suppress the oxidation of lysosomes at physiologically relevant oxygen concentrations. Bilirubin can scavenge the chain-carrying peroxyl radical, which is the primary proposed mechanism of the antioxidant effect.[15] It can act as an important cytroprotector of tissues that are poorly equipped with antioxidant defense systems, including the myocardium and nervous tissue,[16] and it provides important protection against postoperative complications and inflammation.[17] Although the baseline level of bilirubin was slightly higher (but not significant) in group 1, the bilirubin curve was similar in both groups in the 24 hours. However, after 24 hours, the levels were decreased in group 2. The bilirubin levels stayed high in group 1 like other antioxidants.

Uric acid, a metabolic breakdown product of nucleic acids in DNA (purines), is found in the serum at concentrations ten times higher than those of vitamin C and has recently been shown to offer significant antioxidant activity and a potential protecting role against oxidative injury.[18] In contrast to the other antioxidants, the uric acid levels were decreased after 24 hours in group 1. Uric acid, like vitamin C, is known as a low molecular weight antioxidant (LMWA). Decreased levels of uric acid might be related to possible consumption by the myocardium within the period of reperfusion and removal from the circulation.[19]

In both groups, the hs-CRP increase was prompt and peaked within the first 24 hours. After 24 hours, these levels decreased in group 2. In group 1, after the first 24 hours, it showed a slow, sustained increase. These changes may be associated with limited, severe OS in off-pump surgery and were constant in group 1. The higher increase in group 2 versus group 1 may indicate that substantial OS arose after conventional CPB, but this was mainly induced by the surgical trauma.

We think that variations in plasma levels of single antioxidants are not good markers of OS involvement. Indeed, the TAR measurement is a global marker of the antioxidant capacity of plasma. In this prospective clinical study, we tried to point out that the TAR is affected by on-pump and off-pump surgery, and supplementation of antioxidant agents a short time after surgery may lead to desired and beneficial changes in postoperative complications.

To the best of our knowledge, there is no study about changes in serum TAR and its comparison to serum albumin, uric acid, bilirubin, and hs-CRP levels in patients who underwent congenital heart surgery with on-pump or off-pump techniques. One of the limitations of our study was the relatively small sample size of the groups. Another limitation of this study was that the oxidative status of the cyanotic and acyanotic patients did not compare. In a recent study of children with cyanotic or acyanotic congenital heart disease, the level of OS was more evident in the cyanotic group.[20] However, in our study population, the two groups had a fairly homogeneous composition and did not present significant differences concerning the cardiac pathology itself. However, it should be noted that these patients were operated with different surgical techniques. This consideration could affect the patient's response to similar OS. This study showed that there is a strong antioxidant response that occurs after pediatric cardiac surgery in both the off-pump and on-pump techniques, and this response was sustained after the first 24 hours in patients operated with CPB. We think that this result demonstrates the protracted effect of CPB on antioxidant status on the postoperative period. It may be speculated that the hemodilution associated with CPB could be partially responsible for the significant reduction in plasma antioxidative capacity. Finally, it should be noted that the contact between the blood and the CPB circuit is very complex and is related to a number of OS sources. Further studies are necessary to establish whether early and late OS is indeed a cause of CPB-associated postoperative complications in pediatric patients.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Clermont G, Vergely C, Jazayeri S, Lahet JJ, Goudeau JJ, Lecour S, et al. Systemic free radical activation is a major event involved in myocardial oxidative stress related to cardiopulmonary bypass. Anesthesiology 2002;96:80-7.
2. Matata BM, Sosnowski AW, Galiñanes M. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. Ann Thorac Surg 2000;69:785-91.
3. Pyles LA, Fortney JE, Kudlak JJ, Gustafson RA, Einzig S. Plasma antioxidant depletion after cardiopulmonary bypass in operations for congenital heart disease. J Thorac Cardiovasc Surg 1995;110:165-71.
4. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-9.
5. Elmoselhi AB, Lukas A, Ostadal P, Dhalla NS. Preconditioning attenuates ischemia-reperfusion-induced remodeling of Na+-K+-ATPase in hearts. Am J Physiol Heart Circ Physiol 2003;285:H1055-63.
6. Suzuki S, Kaneko M, Chapman DC, Dhalla NS. Alterations in cardiac contractile proteins due to oxygen free radicals. Biochim Biophys Acta 1991;1074:95-100.

7. Biglioli P, Cannata A, Alamanni F, Naliato M, Porqueddu M, Zanobini M, et al. Biological effects of off-pump vs. on-pump coronary artery surgery: focus on inflammation, hemostasis and oxidative stress. Eur J Cardiothorac Surg 2003;24:260-9.
8. Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol 2004;286:R431-44.
9. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001;54:356-61.
10. Gerritsen WB, van Boven WJ, Driessen AH, Haas FJ, Aarts LP. Off-pump versus on-pump coronary artery bypass grafting: oxidative stress and renal function. Eur J Cardiothorac Surg 2001;20:923-9.
11. Akila, D'souza B, Vishwanath P, D'souza V. Oxidative injury and antioxidants in coronary artery bypass graft surgery: off-pump CABG significantly reduces oxidative stress. Clin Chim Acta 2007;375:147-52.
12. Christen S, Finckh B, Lykkesfeldt J, Gessler P, Frese-Schaper M, Nielsen P, et al. Oxidative stress precedes peak systemic inflammatory response in pediatric patients undergoing cardiopulmonary bypass operation. Free Radic Biol Med 2005;38:1323-32.
13. Ballmer PE, Reinhart WH, Jordan P, Bühler E, Moser UK, Gey KF. Depletion of plasma vitamin C but not of vitamin E in response to cardiac operations. J Thorac Cardiovasc Surg 1994;108:311-20.
14. Malatino LS, Benedetto FA, Mallamaci F, Tripepi G, Zoccali C, Parlongo S, et al. Smoking, blood pressure and serum albumin are major determinants of carotid atherosclerosis in dialysis patients. CREED Investigators. Cardiovascular Risk Extended Evaluation in Dialysis patients. J Nephrol 1999;12:256-60.
15. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043-6.
16. Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. Cancer Causes Control 2001;12:887-94.
17. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin Chem 2000;46:1723-7.
18. Becker BF. Towards the physiological function of uric acid. Free Radic Biol Med 1993;14:615-31.
19. Molicki JS, Draaisma AM, Verbeet N, Munneke R, Huysmans HA, Hazekamp MG, et al. Prime solutions for cardiopulmonary bypass in neonates: antioxidant capacity of prime based on albumin or fresh frozen plasma. J Thorac Cardiovasc Surg 2001;122:449-56.
20. Ercan S, Cakmak A, Kösecik M, Erel O. The oxidative state of children with cyanotic and acyanotic congenital heart disease. Anadolu Kardiyol Derg 2009;9:486-90.