



## Comparison of antioxidant reserve capacity of children with acyanotic & cyanotic congenital heart disease

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**Background & objectives:** Oxidative stress can cause many diseases and increases the risk of post-operative complications in children with congenital heart disease. For these reasons, this study was aimed to investigate the differences between cyanotic and acyanotic paediatric patients who underwent heart surgery with markers of oxidative stress.

**Methods:** Eighty five patients were included in the study. The samples taken before the surgery and within the first 24 h after the surgery were evaluated for haemoglobin (Hb), leukocytes, uric acid, glutathione (GSH), malondialdehyde and total antioxidant capacity. Cyanotic, acyanotic, hyperoxygenated, normo-oxygenated, cardiac surgery with or without cardiopulmonary bypass (CPB) comparisons were made.

**Results:** Positive correlation was found between age and pre-operative total antioxidant status values. Cyanotic and acyanotic patients did not have different antioxidant reserve capacities preoperatively. Although pre-operative thiobarbituric acid reactive substances (TBARS) levels were significantly lower in cyanotic patients, post-operative levels were higher. TBARS levels increased and GSH levels reduced postoperatively. The level of oxygenation did not cause a significant difference on markers of oxidative stress. The duration of CPB did not have negative effects on oxidative stress.

**Interpretation & conclusions:** Cyanotic and younger patients were found to be more vulnerable to oxidative stress. The increased levels of TBARS and the decreased levels of GSH could be the indicators of oxidative damage depending on many factors such as surgery, CPB, ischaemia/reperfusion, inflammation, iron overload and oxygenation. The level of oxygenation does not cause a noticeable difference in oxidative stress. CPB causes oxidative stress, but if it is conducted appropriately, the duration of CPB does not cause a significant negative impact on oxidative stress.

**Key words** Acyanotic - cardiopulmonary bypass - congenital heart diseases - cyanotic - oxidative stress - oxygenation

Oxidative stress, which is described as the imbalance between the production of reactive oxygen species (ROS) and biological system's ability of detoxify these reactive metabolites or ability to repair

the damage caused by them, is associated with many diseases<sup>1</sup>. Both cyanotic and acyanotic congenital heart diseases are believed to trigger the pro-oxidant process in the human body<sup>2</sup>. The total antioxidant and oxidant status and oxidative stress index are reported to be increased in children with congenital heart disease<sup>3</sup>. Multiple studies have shown that not only there are distinct differences between cyanotic and acyanotic patients but also the age of the patients, oxygenation level, surgery with or without cardiopulmonary bypass (CPB), CPB duration and haemoglobin (Hb) levels of the patients are emphasized to be important<sup>4-8</sup>.

Total antioxidant capacity gives information about the total capacity of water-soluble antioxidants against oxidative stress, while malondialdehyde (MDA) is an end product of lipid peroxidation. Glutathione (GSH) and GSH associated enzymes are also important because the first step in the protection mechanism against ROS and oxidative stress is GSH and the second step is created by GSH dependent enzymes<sup>9</sup>.

This prospective study was aimed to investigate both the potential differences between cyanotic and acyanotic paediatric patients who underwent heart surgery in terms of oxidative stress markers and also the effects of age, operation type, CPB technique and duration and oxygenation levels on these markers.

### Material & Methods

This prospective, observational study was conducted in consecutive paediatric patients with congenital heart disease who underwent open-heart surgery between November 2014 and February 2015, in the department of Cardiovascular Surgery, Hacettepe University Hospital, Ankara, Turkey. Patients with pre-existing systemic diseases (renal failure or hepatic dysfunction), those who had a local or systemic infection or inflammation, and patients with respiratory failure (on mechanical ventilation) were not included in the study. Patients who died during the immediate post-operative period were also excluded from the study. Three patients were excluded due to the systemic diseases, six for lack of complete data and three patients were excluded due to post-operative mortality. As a result, the study was conducted with the remaining 85 patients. The patients were divided into four groups based on their cyanosis and oxygenation (normo-oxygenation: 80-150 mmHg PaO<sub>2</sub>, hyper-oxygenation: 150-250 mmHg PaO<sub>2</sub>) criteria. The study protocol was approved by the Institutional Ethics Committee

and written informed consent was obtained from the patients.

*Clinical management:* A membrane oxygenator and non-pulsatile roller circuit were used for all patients during the CPB. The CPB circuit was primed with the lactated Ringer solution, sodium bicarbonate, mannitol and heparin. All patients were maintained at 26-32°C while on CPB. Myocardial protection was achieved by using blood cardioplegia solution with a cardioplegia delivery system. Packed red blood cells (PRBCs) were added on CPB circuit to maintain a haematocrit of 30 per cent. Heparin was neutralized with protamine at the conclusion of CPB. Leucocyte depleted PRBCs were transfused in the intensive care unit to maintain Hb levels over 10 g/100 ml. Platelet concentrate was transfused when platelet levels were <100,000/ml. Fresh frozen plasma was transfused for oozing from the surgical site, catheter sites, increased chest tube drainage and prothrombin time or activated partial thromboplastin time >1.5 times normal.

*Sample collection & biochemical parameters:* Samples collected before the surgery and within the first 24 h after the surgery. The MDA levels were measured using thiobarbituric acid reactive substances (TBARS) method and TBARS Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA, Item No. 10009055). All samples were double-checked, loaded in the microplates, read colorimetrically at the absorbance of 530-540 nm and fluorometrically at the excitation wavelength 530 nm and emission wavelength 550 nm in SpectraMax M2, Molecular Devices (Molecular Devices, LLC. San Jose, CA, USA).

The GSH levels were measured using GSH Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA, Catalog No. 703002). All samples were lyophilized (1/3), double-checked, loaded in the microplates and read at the absorbance of 405-414 nm for half an hour, including 5 minutes intervals in SpectraMax M2, Molecular Devices.

The total antioxidant status (TAS) levels were measured using Antioxidant Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA, Item No. 709001). All samples were double-checked, loaded in the microplates, read at the absorbance of 750 nm and 405 nm in SpectraMax M2, Molecular Devices. The results for all the tests were evaluated using the reactions and tables in the kit with SoftMax

Pro 4.8 programme (SoftMax Pro Software Version 4.8, Molecular Devices Corporation, Sunnyvale, CA, USA).

*Other variables:* Age, gender, clinical symptoms, diagnoses of the patients, pre- and post-operative Hb, leucocyte, total protein, albumin, lactate, uric acid, GSH, MDA and TAS levels, surgical procedure, oxygenation level during induction of anaesthesia, presence and duration of CPB and aortic cross-clamping time were all recorded and analyzed.

*Statistical analysis:* Quantitative variables were expressed as mean±standard deviation, median, minimum and maximum. The frequency and percentage distributions of qualitative data were given. Normality evaluation was performed using Kruskal–Wallis and Shapiro–Wilk tests. Qualitative data were compared using the Chi-square test, whereas continuous data comparison among the normal distribution of independent groups was performed by applying independent samples t-test and for dependent groups by applying paired t-test. Mann–Whitney U and Wilcoxon tests were used for the evaluation of independent and dependent groups, respectively. Correlation between continuous variables was analyzed by Spearman correlation. Data were analyzed using SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp).

## Results

Of the 85 patients, 39 (45.8%) were male and 46 (54.2%) were female. The median age was 19.0 months (IQR: 70.5 months; Q1: 9.0 months; Q3: 79.5 months; range, 0.1-504 months). Twenty five patients (29.4%) were aged less than one year. Because oxygenation data could not be obtained for nine patients; evaluation for oxygenation data was made for 76 patients (Table I).

About 72.2 per cent (n=57) of the patients were operated with CPB and 27.8 per cent (n=22) were operated without CPB. Mean CPB time was 84.7 minutes and mean aortic clamp time

was 49.71 min. A statistically significant positive correlation was found between CPB duration and post-operative TAS levels ( $P<0.05$ ). There were no significant correlation between CPB duration and both post-operative TBARS and GSH levels.

Pre- and post-operative albumin, total protein, leucocytes, lactate, Hb and uric acid levels were compared. Post- albumin and total protein levels were significantly decreased while leucocytes and lactate levels were significantly elevated compared to pre-operative period ( $P<0.001$ ). Hb and uric acid levels were not significantly different between pre-operative and post- periods. Pre-operative Hb levels in acyanotic patients were significantly lower than cyanotic patients ( $P<0.01$ ). There was no significant difference in leucocyte levels in samples taken from cyanotic and acyanotic patients in both pre- and post-operative periods. There was no significant difference in Hb levels between pre-operative and post-operative periods in patients with CPB.

*Total antioxidant status (TAS):* There were no significant differences between pre- and post-operative levels of TAS, both in acyanotic and cyanotic groups. In acyanotic hyperoxygenated patients, post-operative TAS levels were significantly lower than pre-operative levels ( $P<0.05$ ). There was no significant difference between pre- and post-operative TAS levels in normo-oxygenated patients. In the cyanotic group, pre-operative and post-operative TAS levels were similar in both hyperoxygenated and normo-oxygenated subgroups (Table II).

*Thiobarbituric acid reactive substances (TBARS):* Post-operative TBARS levels were significantly higher than pre-operative levels ( $P<0.01$ ) in hyperoxygenated group. Pre- and post-operative TBARS levels were similar in hyperoxygenated and normo-oxygenated acyanotic subgroups. Post-operative TBARS levels were significantly higher than pre-operative levels in both the cyanotic group ( $P<0.01$ ), hyperoxygenated ( $P<0.01$ ) and normo-oxygenated ( $P<0.01$ ) cyanotic subgroups (Table III).

*Glutathione (GSH):* Post-operative GSH levels were significantly lower than preoperative levels ( $P<0.05$ ). In acyanotic group, post-operative GSH levels were significantly lower than pre-operative GSH levels ( $P<0.01$ ). Although post-operative GSH levels were significantly lower than pre-operative levels in normo-oxygenated acyanotic patients ( $P<0.05$ ), there

**Table I.** Comparison of the patients for cyanosis and oxygenation status

CHD type	Hyperoxygenated, n (%)	Normo-oxygenated, n (%)
Acyanotic (n=43)	20 (46.5)	23 (53.5)
Cyanotic (n=33)	15 (45.5)	18 (54.5)
Total (n=76)	35 (46.1)	41 (53.9)

CHD, congenital heart defect

**Table II.** Pre-operative and post-operative TAS average values in cyanotic and acyanotic patients according to oxygenation types

CHD type	Oxygenation type	Descriptive statistics	Pre-operative TAS mean	Post-operative TAS mean
Acyanotic	Hyperoxygenated	n, Mean±SD	20, 7.20±1.61	20, 6.36±1.69*
		Median (minimum-maximum)	7.20 (9.57-7.20)	5.92 (9.84-5.92)
	Normo-oxygenated	n, Mean±SD	22, 7.11±1.79	22, 7.12±1.68
		Median (minimum-maximum)	7.09 (4.05-10.21)	6.56 (4.22-10.63)
Cyanotic	Hyperoxygenated	n, Mean±SD	15, 6.96±1.75	15, 7.61±1.62
		Median (minimum-maximum)	6.94 (4.34-9.91)	7.90 (4.81-10.13)
	Normo-oxygenated	n, Mean±SD	16, 6.86±1.72	18, 6.43±1.54
		Median (minimum-maximum)	6.81 (3.73-10.46)	6.22 (3.26-9.09)

\* $P < 0.05$  compared to pre-operative levels; CHD, congenital heart defect

**Table III.** Pre- and post-operative TBARS (MDA) average values in cyanotic and acyanotic patients according to oxygenation types

CHD type	Oxygenation type	Descriptive statistics	Pre-operative TBARS mean	Post-operative TBARS mean
Acyanotic	Hyperoxygenated	n, Mean±SD	19, 22.46±57.70	20, 21.63±57.42
		Median (minimum-maximum)	9.19 (4.96-260.58)	8.68 (3.20-265.19)
	Normo-oxygenated	n, Mean±SD	22, 8.62±2.08	22, 10.81±4.65
		Median (minimum-maximum)	8.32 (5.61-14.15)	9.30 (4.76-23.60)
Cyanotic	Hyperoxygenated	n, Mean±SD	15, 7.81±2.50	15, 18.30±13.34**
		Median (minimum-maximum)	7.37 (4.27-11.98)	12.85 (7.06-52.24)
	Normo-oxygenated	n, Mean±SD	16, 8.91±5.10	18, 23.36±48.99**
		Median (minimum-maximum)	7.67 (5.08-26.87)	10.98 (5.94-218.82)

\*\* $P < 0.01$  compared to pre-operative levels; CHD, congenital heart defect

was no significant difference between pre-operative and post-operative GSH levels in hyperoxygenated acyanotic patients. In cyanotic patients, pre- and post-operative GSH levels were similar. There was no significant difference between pre- and post-operative GSH levels both in hyperoxygenated and normo-oxygenated cyanotic patients (Table IV).

### Discussion

Clinical outcomes for children with cyanotic congenital heart disease are known to be worse than those with acyanotic ones<sup>10-12</sup>. This may be caused by the lack of the antioxidant capacity of these children and besides, they are vulnerable to the oxidative stress and the formation of protein carbonylation and lipid peroxidation due to hypoxia<sup>11</sup>. Rokicki *et al*<sup>10</sup> investigated the relationship between congenital heart diseases and oxidative stress but failed to show any significant difference compared with the healthy control group. Allen *et al*<sup>12</sup> demonstrated that there were no significant differences between cyanotic and acyanotic infants regarding their antioxidant reserve

capacities during the pre-operative period. Ercan *et al*<sup>13</sup> showed that although oxidative stress index values (TOS, TAS and OSI) were significantly higher in cyanotic group than acyanotic and control groups, there was no significant difference between the control group and acyanotic patients. Since TAS levels reflect the spectrum of general antioxidant activity formed against different reactive oxygen/nitrogen radicals, reduction of these levels suggest an increase in free radicals which cause oxidative stress<sup>11</sup>.

In this study, the TAS levels were compared and no significant difference was found between pre- and post-operative levels in cyanotic and acyanotic patient groups as well as hyperoxygenated and normo-oxygenated patients. Although TAS levels were similar during pre- and post-operative periods in both hyperoxygenated and normo-oxygenated cyanotic patients, post-operative TAS levels were significantly lower in hyperoxygenated acyanotic patients. Furthermore, a proportional relationship between TAS levels and the age of the patients was found. Thus, it may be speculated that younger patients

**Table IV.** Pre- and post-operative glutathione (GSH, nmol/ml) average values in cyanotic and acyanotic patients according to oxygenation types

CHD type	Oxygenation type	Descriptive statistics	Pre-operative GSH endpoint mean	Post-operative GSH endpoint mean
Acyanotic	Hyperoxygenated	n, Mean±SD	20, 0.90±2.91	20, 0.07±2.06
		Median (minimum-maximum)	-0.30 (-1.99-8.03)	-0.43, -2.00-6.66)
	Normo-oxygenated	n, Mean±SD	22, 0.69±2.27	22, -0.99±1.86*
		Median (minimum-maximum)	0.04 (-1.66-7.70)	-0.63 (-6.53-2.26)
Cyanotic	Hyperoxygenated	n, Mean±SD	15, 3.06±8.00	15, 1.52±3.00
		Median (minimum-maximum)	0.11 (-1.84-28.40)	0.72 (-1.91-8.67)
	Normo-oxygenated	n, Mean±SD	18, -0.24±2.25	18, -0.84±2.46
		Median (minimum-maximum)	-0.85 (-2.75-7.17)	-1.00 (-8.19-3.03)

\* $P < 0.05$  compared to pre-operative levels; CHD, congenital heart defect

are more vulnerable to oxidative stress and oxidative damage.

Lipid peroxidation and protein oxidation which are displayed with MDA and protein carbonyl levels in the blood are other markers of oxidative stress<sup>14,15</sup>. The oxidation of cell membrane lipids exposes aldehydes, the TBARS<sup>16</sup>. TBARS is a general, non-specific marker of oxidative damage<sup>17</sup>.

Pirincioglu *et al*<sup>11</sup> compared patients with congenital heart disease with a control group and showed that both MDA and PCO levels were significantly higher in patients with congenital heart disease being higher in cyanotic ones. In this study, lipid peroxidation was evaluated through TBARS and post-operative levels were found to be significantly higher than pre-operative levels. There were no significant differences between pre- and post-operative levels in acyanotic patients. However, post-operative levels were significantly higher than pre-operative levels in cyanotic patients. Although there was no significant difference between pre- and post-operative levels of TBARS in hyperoxygenated patients, post-operative values were significantly higher in normo-oxygenated patients. In cyanotic patients, both hyperoxygenated and normo-oxygenated, post-operative TBARS levels were significantly higher than pre-operative levels. Therefore, hyperoxygenation may not have a significant influence on oxidative stress in acyanotic patients.

Rokicki *et al*<sup>10</sup> showed that GSH peroxidase levels were lower in patients with cyanotic congenital heart diseases than acyanotic patients and control group. Our study showed that post-operative GSH levels

were significantly lower than pre-operative levels in acyanotic patients, while there was no significant difference between pre- and post-operative values of cyanotic patients.

Ischaemia-reperfusion injury is common after paediatric heart surgery performed with CPB. Oxidative stress followed by CPB may primarily be due to haemolysis rather than inflammation or reperfusion<sup>18</sup>. Another cause of oxidative stress may be plasma redox-active iron (Hb released from lysed RBCs)<sup>19</sup>. Free iron contributes to oxidative stress hours after termination of CPB. However, Hb itself is considered to be the main reason for early perioperative oxidative stress. Hb can also catalyze oxidative reactions<sup>20</sup>. Prolongation of CPB time is found to be associated with increased oxidative stress<sup>7,8</sup>. Calza *et al*<sup>2</sup> showed that oxidative stress markers were high even before the commencement of CPB in children with congenital heart disease. Bulutcu *et al*<sup>21</sup> found no significant differences between cyanotic and acyanotic patients' MDA levels before CPB. However, MDA levels after CPB were significantly higher in cyanotic patients in that study. In the present study, there were no significant differences between pre- and post-operative levels of TAS, TBARS, and GSH in patients who underwent CPB, however, there was a linear relationship between CPB time and TAS levels.

Del Nido *et al*<sup>22</sup> have identified the lipid peroxidation and reoxygenation injury occur following repair of Fallot tetralogy. Allen *et al*<sup>12</sup> stated that free radical production could be limited by reducing the oxygen concentration in the CPB system. During the early stages of CPB, lipid peroxidation which is

caused by free radicals occurs as a result of ischaemia. However, effective CPB management reduces the amount of oxidative products to normal levels in a short period<sup>23</sup>.

As endogenous tissue stores of antioxidants are similar between acyanotic and cyanotic hearts before CPB, sudden reoxygenation of chronic hypoxic infants may cause a higher production of free oxygen radicals<sup>12</sup>. The first clinical evidence of reoxygenation damage caused by oxygen was demonstrated by Allen *et al*<sup>12</sup> and Bulutcu *et al*<sup>21</sup>. They showed that sudden reoxygenation during hyperoxic CPB caused a significant decrease in an antioxidant reserve capacity in cyanotic infants by MDA levels. Large amount of free radicals were found to be released in the first 10-20 min of CPB. However, the same hyperoxia levels caused minimal changes at the antioxidant levels of acyanotic patients. Teoh *et al*<sup>24</sup> described that the pre-operative exposure to partial oxygen pressure is associated with a reduction in endogenous antioxidant content. Thus, oxidative damage can significantly be reduced by applying the CPB with lower PaO<sub>2</sub> (partial pressure of oxygen)<sup>12,21</sup>.

One of the limitations of this study was the relatively small sample size of the groups. Variability in the age of the patients and incomplete laboratory results of some patients were another limitation of our study. Besides blood samples at specific time points would have been more appropriate.

In conclusion, our results show that (i) cyanotic and younger patients are more vulnerable to oxidative stress; (ii) increased levels of TBARS and the decreased levels of GSH are the indicators of oxidative damage; (iii) oxidative damage depends on many factors such as surgery, CPB, ischaemia/reperfusion, inflammation, iron overload and oxygenation; (iv) as effective CPB management reduces the amount of oxidative products, prolongation of CPB time does not create a significant negative impact on oxidative stress.

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**Conflicts of Interest:** None.

## References

- Toro J, Rodrigo R. Oxidative stress: Basic overview. In: Oxidative Stress and Antioxidants. *World Allergy Organ J* 2009; 1 : 1-12.
- Calza G, Lerzo F, Perfumo F, Borini I, Panizzon G, Moretti R, *et al*. Clinical evaluation of oxidative stress and myocardial reperfusion injury in pediatric cardiac surgery. *J Cardiovasc Surg* 2002; 43 : 441-7.
- Manso HP, Carmona F, Dal-Pizzol F, Petronilho F, Cardoso F, Castro M, *et al*. Oxidative stress markers are not associated with outcomes after pediatric heart surgery. *Pediatr Anesth* 2013; 23 : 188-94.
- Ferrari R, Alfieri O, Curello S, Ceconi C, Cargnoni A, Marzollo P, *et al*. Occurrence of oxidative stress during reperfusion of the human heart. *Circulation* 1990; 81 : 201-11.
- Laffey JG, Boylan JF, Cheng DC. The systemic inflammatory response to cardiac surgery: Implications for the anesthesiologist. *Anesthesiology* 2002; 97 : 215-52.
- Mumby S, Chaturvedi RR, Brierley J, Lincoln C, Petros A, Redington AN, *et al*. Iron overload in paediatrics undergoing cardiopulmonary bypass. *Biochim Biophys Acta* 2000; 1500 : 342-8.
- 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.
- McColl AJ, Keeble T, Hadjinikolaou L, Cohen A, Aitkenhead H, Glenville B, *et al*. Plasma antioxidants: Evidence for a protective role against reactive oxygen species following cardiac surgery. *Ann Clin Biochem* 1998; 35(Pt 5) : 616-23.
- Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 1999; 31 : 273-300.
- Rokicki W, Strzałkowski A, Kłapcińska B, Danch A, Sobczak A. Antioxidant status in newborns and infants suffering from congenital heart defects. *Wiad Lek* 2003; 56 : 337-40.
- Pirincioglu AG, Alyan O, Kizil G, Kangin M, Beyazit N. Evaluation of oxidative stress in children with congenital heart defects. *Pediatr Int* 2012; 54 : 94-8.
- Allen BS, Rahman S, Ilbawi MN, Kronon M, Bolling KS, Halldorsson AO, *et al*. Detrimental effects of cardiopulmonary bypass in cyanotic infants: Preventing the reoxygenation injury. *Ann Thorac Surg* 1997; 64 : 1381-7.
- 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.
- Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; 52 : 601-23.
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, *et al*. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990; 186 : 464-78.
- Lefter DJ, Granger DN. Oxidative stress and cardiac disease. *Am J Med (Review)* 2000; 109 : 315-23.
- Halliwell B, Gutteridge J. *Free radicals in biology and medicine*. 4<sup>th</sup> ed. Oxford: Oxford University Press; 2007.
- Lull ME, Salli CN, Freeman WM, Myers JL, Midgley FM, Thomas NJ, *et al*. Plasma biomarkers in pediatric patients

- undergoing cardiopulmonary bypass. *Pediatr Res* 2008; 63 : 638-44.
19. Ihnken K, Morita K, Buckberg GD, Sherman MP, Young HH. Studies of hypoxemic/reoxygenation injury: Without aortic clamping. III. Comparison of the magnitude of damage by hypoxemia/reoxygenation versus ischemia/reperfusion. *J Thorac Cardiovasc Surg* 1995; 110 : 1182-9.
  20. Everse J, Hsia N. The toxicities of native and modified hemoglobins. *Free Radic Biol Med* 1997; 22 : 1075-99.
  21. Bulutcu FS, Bayindir O, Polat B, Yalcin Y, özBek U, Cakali E. Does normoxemic cardiopulmonary bypass prevent myocardial reoxygenation injury in cyanotic children? *J Cardiothorac Vasc Anesth* 2002; 16 : 330-3.
  22. Del Nido RJ, Mickle DA, Wilson GJ, Benson LN, Weisel RD, Coles JG, *et al.* Inadequate myocardial protection with cold cardioplegic arrest during repair of tetralogy of Fallot. *J Thorac Cardiovasc Surg* 1988; 95 : 223-9.
  23. Ulus AT, Aksoyek A, Ozkan M, Katircioglu SF, Basu S. Cardiopulmonary bypass as a cause of free radical-induced oxidative stress and enhanced blood-borne isoprostanes in humans. *Free Radical Biol Med* 2003; 34 : 911-7.
  24. Teoh KH, Mickle DA, Weisel RD, Li RK, Tumiati LC, Coles JG, *et al.* Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. *J Thorac Cardiovasc Surg* 1992; 104 : 159-64.

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