Original Article

Assessment of Antioxidant System Status before and after Operation in Neonates with Congenital Heart Disease

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ABSTRACT

Background/Aim: Oxidative stress is considered to have a significant role in the development of cardiovascular diseases (CVDs) as well as many other diseases. Therefore, the purpose of the study is to evaluate the antioxidant system status at pre- and post-operative period in newborns with congenital heart disease (CHD) requiring operation. **Materials and Methods:** Fifty CHD patients participated in this research. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thiobarbituric acid reactive substances (TBARS) levels were studied in blood samples. RACHS-1 score, blood lactate levels, and hypoxic events were also recorded. Comparisons of antioxidant system parameters were conducted at pre- and post-operative periods and also between exitus and discharged groups. **Results:** GPx activity and TBARS levels were significantly higher in the pre-operative period than post-operative period though the other antioxidant enzymes were not altered. In pre-operative period, GPx activity was low in addition to rarer hypoxic events in the discharged group. Also, a negative correlation was found between SOD and GPx activities in pre-operative period. **Conclusion:** The results provide fundamental data showing the lowered GPx activity and TBARS levels considered as sensitive oxidative biomarkers after the operation. It was assumed that antioxidant system parameters might show changes after the operation, and GPx is prominent for resistance to hypoxic conditions. Post-operative reduction of GPx and TBARS levels is significant for evaluating the antioxidant system alterations after the operation. However, further investigations follow long-term duration for post-operative monitoring to estimate how antioxidant system status changes to improve the treatment of the health condition.

KEYWORDS: *Antioxidant enzymes, congenital heart disease, newborns, oxidative stress*

INTRODUCTION

Neonates with critical CHD are exposed to excess oxygen radicals due to ischemia, reperfusion, and chronic hypoxia, which cause deterioration in the patient's condition leading to difficulties in treatment.[1] CHD and the operation procedure of CHD might affect the oxidative status of the patients.

Oxidative stress is considered to play a particularly important role in cardiovascular pathology in adults.[2-4] The role of reactive oxygen species (ROS) in cardiovascular diseases (CVDs) is well-documented.[5-7] All aerobic organisms possess complex enzymes known as antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and non-enzymatic antioxidants like vitamins E and C, nitric oxide, iron, and copper to cope with the oxidative stress caused by ROS.[8]

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To our best knowledge, there are only a few reports in the literature concerning the role of oxidative stress in children with a CHD.[9,10] Previous researches were conducted to compare the antioxidant reserve capacity between cyanotic and acyanotic CHD in children and resulted with a significantly higher level of oxidative stress in cyanotic CHD than in the acyanotic CHD.[10,11] In another study, children at high risk of having CHD showed a significant decrease in the levels of GPx and CAT activities when compared to the non-risky control group.[12] Nevertheless, it has gained more significance to evaluate the response of the first-line defense antioxidant enzyme activities than total antioxidant status to see how they compensate for the imbalanced antioxidant status, specifically.

It becomes more important to investigate the clinical outcomes for defining the oxidative stress role in neonates with CHD due to insufficient data about the oxidative stress impact on the immature heart.[13] In this sense, it was hypothesized the status of the antioxidant system could be differed in the patients in the pre- and post-operative when they were stable due to their variability of oxygen insufficiency. Therefore, it was aimed to evaluate the antioxidant system status at pre- and post-operative period in newborns with congenital heart disease (CHD) requiring operation.

Materials and Methods

The present study includes patients who underwent surgery due to cyanotic CHD between 2018 and 2019 in the neonatal intensive care unit (NICU) of Çukurova University Faculty of Medicine. The study was approved by the Çukurova University Scientific Research Community Ethics Committee.

All patients who underwent surgery due to cyanotic CHD during study period were included. Patients with intrauterine infection, congenital or chromosomal anomalies, systemic infection requiring inotropic therapy before operation were excluded.

Antioxidant enzymes: SOD, CAT, and GPx activities and TBARS levels for lipid peroxidation before the operation and at 8 hours after the operation were measured and compared.

During the post-operative period, blood gas measurements were made hourly in first 4 hours and then 2 to 4 hours apart in the first 24 hours. Capillary/venous blood gases of the patients was measured 3–6 times in first 24 hours using ABL 800 FLEXR blood device, and those with lactate levels above 2.5 mmol/L were considered as abnormal.

"RACHS-1" classification, which is one of the risk assessment systems in congenital heart surgery, was used to determine the morbidity and mortality of the patients. Risk score of 1 was the lowest and 6 was the highest.[14]

Increased creatinine level was defined as a 50% or more increase in serum creatinine level (1.5 times baseline), and anuria/oliguria was defined as a decrease in urine output of less than 0.5 mL/kg per hour for more than 6 hours.[15]

The need for reoperation in the last 24 hours, a history of arrest during the operation, high creatine level, and lactate 2.5 mmol/L were evaluated as prognostic factors. Exposure to hypoxic events was evaluated according to the presence of cardiac arrest, resuscitation need, circulatory disorder, arrhythmia, and convulsion.

Antioxidant parameter analysis was performed as stated in the study of Koc *et al*.[16]

Statistical analysis

IBM SPSS Statistics Version 20.0 package program was used for statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements as mean and standard deviation (median and minimum–maximum where necessary). Chi-square test statistics were used to compare categorical measurements between groups. In the comparison of numerical measurements between groups, Student's *t*-test was used in independent groups if assumptions were met, and Mann–Whitney *U* test was used if assumptions were not provided. In the comparison of dependent numerical measurements, *t*-test was used in dependent groups. To examine the interaction of numerical measurements with each other, the Pearson correlation coefficient and the corresponding *P* value were obtained. The statistical significance level was taken as 0.05 in all tests.

Results

Our prospective study consists of 50 patients who were diagnosed with cyanotic CHD in CUTF-NICU between 2018 and 2019. The study group was diagnosed during the fetal period and after birth by the same cardiologist.

Arterial switch operation was performed in 12 (24%) of 50 operated patients, shunt operation in 13 (26%), pulmonary arterial band operation in 13 (26%). Hypoplastic left heart syndrome, valve valvuloplasty were applied to the remaining 12 (24%) patients. Demographic and clinical properties of the patients were in Table 1.

Figure 1 showed the comparison of antioxidant enzyme levels of pre-operative and post-operative groups. According to data, GPx activity [Figure 1]

and TBARS levels [Figure 2] were statistically significantly lower when compared with the pre-operative and post-operative groups ($P < 0.05$). TBARS levels decreased as 14% and GPx decline was 25% after post-operation in contrast to pre-operative period. However, there were not any significant changes in other enzyme activities and the protein levels ($P > 0.05$) [Figures 1 and 3].

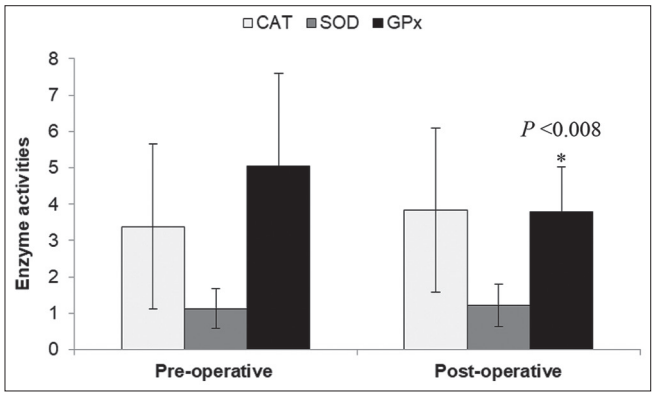

Figure 1: Activities of CAT (µmol H_2O_2/mg prot/min), SOD (U/mg prot), and GPx (µmol/mg prot/min) in the pre- and post-operative groups of cyanotic CHD patients. Asterisks indicate the statistical differences between the groups ($P < 0.05$)

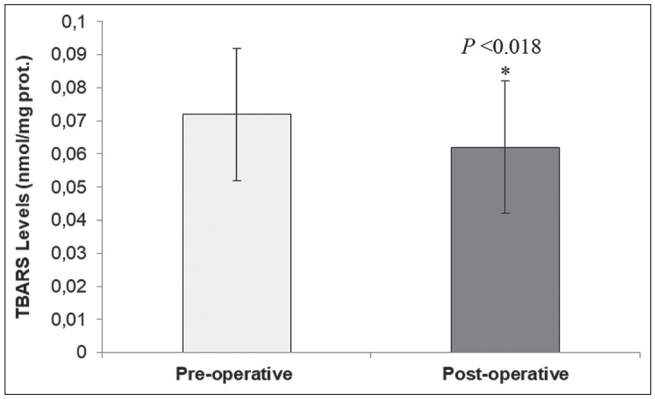

Figure 2: TBARS levels in the pre- and post-operative groups of cyanotic CHD patients

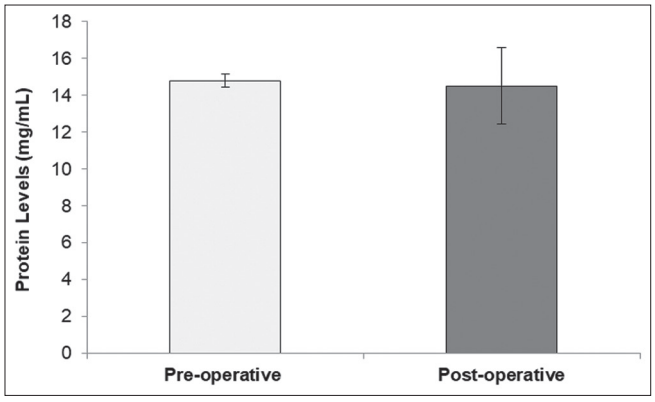

Figure 3: Protein levels in the pre- and post-operative groups of cyanotic CHD patients

The antioxidant enzyme levels and lipid peroxidation levels were compared in patients who were discharged and who died. As shown in Table 2, pre-operative Gpx levels were higher in the exitus group before operation; however, there was no difference after operation ($P < 0.05$). Also, a number of patients with

Table 1: Demographic characteristics of the study groups

Demographic characteristics	Study group (*n*=50)
Gestational (wk) mean±SD (Min-Max)	38.02±1.70 (34-42)
Birth weight (g) mean±SD (Min-Max)	2981.3±541.8 (1780-4550)
Gender Male, *n* (%)	33 (66)
Mode of delivery C/S, *n* (%)	19 (38)
Clinical Features	
Apgar 1th min mean±SD (Min-Max)	6.72±1.47 (3-9)
Apgar 5th min mean±SD (Min-Max)	8.12±1.05 (5-10)
RACHS-1 score mean±SD (Min-Max)	4.06±1.25 (2-6)
Postnatal age of operation (days) mean±SD (Min-Max)	7.2±5.4 (1-23)
Post-operative lactate ≥2.5 mmol/L, *n* (%)	31 (62%)
Reoperation need in 24 hours after operation, *n* (%)	7 (14%)
Severe bleeding (>10 ml/kg/h), *n* (%)	21 (42%)
Post-operative Infection in first week, *n* (%)	19 (38%)
Exitus due to cardiac etiology, *n* (%)	18 (36%)

Table 2: Comparison of the clinical status of Group 1 (Exitus) and Group 2 (Discharged) patients. Significant differences were in bold (*P*<0.05)

Parameters	Group 1 (*n*=18) Exitus	Group 2 (*n*=32) Discharged	*P*
TBARS-preop	0.071±0.02	0.072±0.02	0.794
TBARS-postop	0.059±0.02	0.063±0.02	0.499
CAT-preop	3.30±2.08	3.48±2.45	0.767
CAT-postop	2.83±1.95	3.82±2.24	0.251
SOD-preop	1.11±0.41	1.15±0.62	0.756
SOD-postop	1.04±0.39	1.36±0.66	0.109
GPx-preop	6.15±2.50	4.46±2.36	0.009
GPx-postop	3.98±1.03	3.67±1.36	0.502
RACHS-1 score	4.42±1.30	3.94±1.13	0.180
	***n* (%)**	***n* (%)**	
Preop hypoxic event	12 (66.6)	11 (34.3)	0.040
Lactate (postop 8th hour)			0.006
<2,5 mmol/L	3 (16.6)	16 (50)	
≥2,5 mmol/L	15 (83.4)	16 (50)	

Table 3: Correlation results among the antioxidant parameters of pre- and post-operative groups

Time	Parameters	Correlation coefficients	Protein	TBARS	CAT	SOD	GPx
Pre-operative	Protein	r^2		-0.157	0.096	-0.170	0.012
		P		0.304	0.620	0.335	0.950
	TBARS	r^2			-0.023	-0.072	0.036
		P			0.865	0.560	0.784
	CAT	r^2				0.126	-0.157
		P				0.414	0.354
	SOD	r^2					-0.439**
		P					0.002
Post-operative	Protein	r^2		-0.081	-0.194	0.123	-0.168
		P		0.602	0.333	0.496	0.342
	TBARS	r^2			0.124	-0.272	-0.023
		P			0.538	0.125	0.902
	CAT	r^2				-0.046	-0.115
		P				0.853	0.660
	SOD	r^2					-0.304
		P					0.158

lactate level >2.5 mmol/l at 8th hour post-operatively were higher in the exitus group.

Discussion

This study demonstrated a significant decrease in GPx activity accompanied by lipid peroxidation decline after the cardiac operation for up to 8 hours in neonates. Based on this, it can be concluded that GPx as one of the first-line antioxidant defense could be a potential biomarker in the evaluation of health status in CHD patients following an operation. A negative correlation between SOD and GPx activities may also support this [Table 3]. In the pre-operative group this negative correlation may be related to the H_2O_2 concentration, the product of the reaction catalyzed by SOD, and a substrate for GPx.

In the present study, GPx activity was significantly reduced in post-operative group in contrast to pre-operative ones. TBARS levels were also accompanied by this situation. GPx is a significant sensitive biomarker since its activity is important for adaptation to hypoxic conditions. The post-operative reduction in the GPx activity might show that oxidative conditions were eliminated with the decline of TBARS levels. In addition, GPx activity was also high in the exitus group compared to the discharged in the pre-operative group. This might be associated with the hypoxic event in this group despite no direct correlation found between these biomarkers. It may conclude that GPx is prominent for resistance to hypoxic conditions and post-operative reduction is significant for determining the hypoxic conditions which is also supported by the TBARS reduction. This situation should also be taken into account due to the lack of data to enlighten the oxidative stress mechanism.

CHD is the most common congenital anomaly in the neonatal period and is responsible for 28% of all major congenital anomalies.[17] Enzymatic and non-enzymatic ROS source activation associated with the redox-sensitive transcriptional factors modulation is involved in the oxidative effects which can cause cardiovascular impairment. It is known that oxidative stress is implicated among the pathophysiological mechanisms of cardiotoxicity.[18]

In the study of Ercan *et al*.,[10] the reason for the insignificant difference between cyanotic and acyanotic patients' post-operative total antioxidant and total oxidant levels may be due to having similar total antioxidant and total oxidant levels in the pre-operative period and the CPB management, with controlled oxygenation, cooling and warming periods, and the use of blood cardioplegia. Total antioxidant status levels significantly increased 24 hours after surgery in the cyanotic patient group. This increase may be because of intensive care unit interventions, thermal instability, and use of blood products, because there was no difference in the post-operative 1st-hour total antioxidant status levels.[19]

In the study by Pirinccioglu *et al.*, total antioxidant status levels in a group of patients with CHD were significantly higher compared with the control group.[20] Elevated malondialdehyde levels presenting the lipid peroxidation in CHD patients are considered as ROS-mediated damage in membranes causing deleterious effects including the reduction in myocardial function. These levels were significantly higher in cyanotic patients

who possess poorer clinical outcomes compared with acyanotic ones. This may result in hypoxia, which is expected to consume the antioxidant reserve capacity and hence leads to a greater susceptibility to oxidative stress.[20] However, in the current study, antioxidant enzymes and TBARS instead of total antioxidant capacity were evaluated to see the specific antioxidant parameter responses and found a difference between pre- and post-operative groups.

On the other hand, Temel *et al*.[11] found similar total antioxidant status, increased TBARS, and unchanged glutathione levels between pre- and post-operative cyanotic groups. Calza *et al*.[21] reported the high oxidative stress marker even before the CBP commencement in children with CHD. However, GPx and TBARS levels were higher at 8th hour after the operation, according to our study. These differences could occur possibly due to measuring the specific antioxidant biomarker levels instead of total antioxidant status and also the time variation after the operation. As a result, the operations seemed successful since the GPx and TBARS values decreased to their normal values in the present data. Therefore, GPx activity and linked with TBARS levels can provide useful information for recovery period. These results emphasized the requirement of further research in the evaluation of antioxidant system parameter responses at different time intervals upon operation with a longer period. In relation to this, Escobar-Diaz *et al*.[22] also demonstrated the increased oxidative stress biomarkers due to hypoxemia and hypoperfusion which may alter the redox balance leading to oxidative stress in fetuses with CHD.

Conclusion

We aimed to investigate how antioxidant system status alters after operation in these patient groups in the sense of insufficient data in the literature. The results also provide fundamental data showing the lowered GPx activity not only after the operation but also in the discharged groups of the pre-operative patients due to its prominent response. It was assumed that antioxidant system parameters might show changes after the operation since hypoxic conditions still exist. In addition, further investigations conduct long-term duration for post-operative monitoring to estimate how antioxidant system status changes to improve the treatment of the health condition.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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