Turkish Journal of Thoracic and Cardiovascular Surgery 2023;31(4):454-466

ORIGINAL ARTICLE / ÖZGÜN MAKALE

Effect of cardiopulmonary bypass on thiol/disulfide homeostasis in congenital heart surgery

Doğumsal kalp cerrahisinde kardiyopulmoner baypasın tiyol/disülfid homeostazisi üzerine olan etkisi

Murat Tanyildiz[1], Ayse Filiz Yetimakman[2], Mutlu Uysal Yazici[2], Ulas Kumbasar[3], Murat Alisik[4], Sinem Oguz[1], Mehmet Bicer[5], Benan Bayrakci[2], Ozcan Erel[4]

Institution where the research was done:
Koç University School of Medicine, Istanbul, Türkiye

Author Affiliations:
[1]Department of Pediatric Intensive Care, Koç University School of Medicine, Istanbul, Türkiye
[2]Department of Pediatric Intensive Care, Hacettepe University School of Medicine, Ankara Türkiye
[3]Department of Cardiovascular Surgery, Hacettepe University School of Medicine, Ankara Türkiye
[4]Department of Medical Biochemistry, Ankara Yıldırım Beyazıt University School of Medicine, Ankara, Türkiye

ABSTRACT

Background: This study aims to investigate whether thiol/disulfide homeostasis parameters measurements could be used as a new biomarker to predict the pre- and post-cardiopulmonary bypass oxidative status of pediatric patients undergoing congenital heart surgery.

Methods: A total of 40 children with congenital heart disease (17 males, 23 females; mean age: 39.6±40.0 months; range, 2 to 216 months) who underwent open-heart surgery were included. The control group consisted of 40 age- and sex-matched healthy children (18 males, 22 females; mean age: 42.8±46.6 months; range, 12 to 156 months). The patients with congenital heart disease were divided into two groups as cyanotic patients (n=18) and acyanotic patients (n=22). Thiol/disulfide parameters were compared among the cyanotic, acyanotic congenital heart disease patients, and control group preoperatively (pre-CPB). The effects of cardiopulmonary bypass on thiol/disulfide parameters, pre-CBP, immediately after cardiopulmonary bypass (post-CPB0), and 24 h after cardiopulmonary bypass (post-CPB24) were investigated.

Results: The mean native and total thiol levels in the cyanotic patients were significantly lower than those in the acyanotic patients and control group (p<0.0001). The cyanotic group exhibited higher disulfide levels than the acyanotic group (p<0.01). The mean native thiol and total thiol levels significantly decreased in the post-CPB0 (p<0.0001). The mean disulfide levels significantly increased in the post-CPB0 than the pre-CPB values (p<0.001). Post-CPB24 native and total thiol levels were elevated compared to post-CPB0 (p<0.0001). The mean disulfide levels significantly increased in the post-CPB24 period than the post-CPB0 values (p<0.001). The survivor patients responded better to oxidative stress than non-survivor patients.

Conclusion: Thiol/disulfide measurement is a promising biomarker in determining the pre- and post-cardiopulmonary bypass oxidative status of pediatric patients undergoing congenital heart surgery. The interpretation of thiol/disulfide levels, pre- and postoperatively, may be used in predicting mortality and outcomes of these patients earlier.

Keywords: Cardiopulmonary bypass, congenital heart defect, oxidative stress, thiol/disulfide.

ÖZ

Amaç: Bu çalışmada tiyol/disülfid homeostazı parametrelerinin ölçümünün doğumsal kalp cerrahisi geçiren pediatrik hastalarda kardiyopulmoner baypas öncesi ve sonrası oksidatif durumu öngörmede yeni bir biyobelirteç olarak kullanılabilip kullanılamayacağı araştırıldı.

Çalışma planı: Açık kalp cerrahisi yapılan doğumsal kalp hastalığı olan toplam 40 çocuk (17 erkek, 23 kız; ort. yaş: 39.6±40.0 ay; dağılım, 2-216 ay) çalışmaya alındı. Kontrol grubu yaş ve cinsiyet ile eşleştirilmiş 40 sağlıklı çocuktan (18 erkek, 22 kız; ort. yaş: 42.8±46.6 ay; dağılım, 12-156 ay) oluşuyordu. Doğumsal kalp hastalığı olan hastalar siyanotik olanlar (n=18) ve asiyonatik olanlar (n=22) olmak üzere iki gruba ayrıldı. Ameliyat öncesinde (KPB öncesi) siyanotik, asiyanotik doğumsal kalp hastalığı olan hastalar ve kontrol grubu arasında tiyol/disülfid parametreleri karşılaştırıldı. Kardiyopulmoner baypasın tiyol/disülfid parametrelerin üzerine etkileri KPB öncesi, kardiyopulmoner baypastan hemen sonra (KPB0 sonrası) ve kardiyopulmoner baypastan 24 saat sonra (KPB24 sonrası) incelendi.

Bulgular: Ortalama native ve total tiyol düzeyleri siyanotik hastalarda asiyanotik hastalar ve kontrol grubuna kıyasla anlamlı düzeyde daha düşük bulundu (p<0.0001). Disülfid düzeyleri siyanotik hastalarda asiyanotik hastalara kıyasla daha yüksek idi (p<0.01). Ortalama native ve total tiyol düzeyleri KPB0 sonrasında anlamlı düzeyde azaldı (p<0.0001). Ortalama disülfid düzeyleri KPB öncesi değerlere kıyasla KPB0 sonrası dönemde anlamlı düzeyde arttı (p<0.001). KPB24 sonrası dönemde native ve total tiyol düzeyleri, KPB0 sonrasına kıyasla artış gösterdi (p<0.0001). Ortalama disülfid düzeyleri, KPB0 sonrası değerlere kıyasla KPB24 sonrası dönemde anlamlı düzeyde arttı (p<0.001). Sağkalan hastalar, kaybedilen hastalara kıyasla oksidatif strese daha iyi yanıt verdi.

Sonuç: Tiyol/disülfid ölçümü doğumsal kalp cerrahisi geçiren çocuk hastalarda kardiyopulmoner baypas öncesi ve sonrası oksidatif durumu belirlemede umut verici bir biyobelirteçtir. Tiyol/disülfid düzeylerinin ameliyat öncesi ve sonrası yorumlanması, bu hastaların mortalite ve sonuçlarının erken öngörülmesinde kullanılabilir.

Anahtar sözcükler: Kardiyopulmoner bypass, doğumsal kalp hastalığı, oksidatif stres, tiyol/disülfid.

Corresponding author: Murat Tanyildiz.
E-mail: murattanyildiz@gmail.com
Doi: 10.5606/tgkdc.dergisi.2023.25283

Received: June 11, 2023
Accepted: August 22, 2023
Published online: October 19, 2023

Cite this article as: Tanyildiz M, Yetimakman AF, Yazici MU, Kumbasar U, Alisik M, Oguz S, et al. Effect of cardiopulmonary bypass on thiol/disulfide homeostasis in congenital heart surgery. Turk Gogus Kalp Dama 2023;31(4):454-466. doi: 10.5606/tgkdc.dergisi.2023.25283.

Among all congenital defects, the primary cause of mortality in pediatric age groups is congenital heart disease (CHD).[1] Many cardiac operations in these patients are performed under cardiopulmonary bypass (CPB) pump with extracorporeal circulation. The oxidative stress response in patients undergoing CPB is associated with worse outcomes and higher mortality, if the organism's antioxidant capacity cannot be overcome with the reactive oxidative species.[2] Cardiopulmonary bypass has been demonstrated to activate multiple signaling cascades, resulting in ischemia-reperfusion injury. Nevertheless, several factors additionally influence the patient's oxidative stress response. These factors encompass the pathophysiological characteristics of the underlying cardiac disease (cyanotic or acyanotic), hyperoxia, and the inflammatory reaction attributed to the artificial surfaces of extracorporeal devices.[3] The interpretation of oxidative status pre- and postoperatively may indicate the mortality and outcomes of the CHD patients earlier.

Thiol (R-SH) groups of proteins are organic molecules containing a sulfhydryl group (-SH) which can undergo oxidation processes and form reversible disulfide (R-SS) bonds with reactive oxidant molecules (Figure 1).[4] Recently, the thiol/disulfide homeostasis (TDH) parameters have been considered as an oxidative stress indicator. Several studies have used TDH parameters as oxidative stress markers in cardiovascular diseases.[5-10] Erel and Erdoğan[11] showed that the levels of total thiol and native thiol were the indicators of the body's antioxidant reserve capacity. In contrast, disulfide parameters reflect the body's response to oxidative stress.[11]

In the present study, we aimed to evaluate the dynamic TDH levels in pediatric patients with CHD and compare these levels with those of the general pediatric population. Additionally, by establishing the oxidative status of CHD patients before and after CPB using TDH parameters, we aimed to investigate the potential correlation between the patients' prognosis and outcomes and their pre- and postoperative TDH parameters.

PATIENTS AND METHODS

This two-center, prospective cohort study was conducted at the cardiac intensive care units (CICUs) of Koc University Hospital, School of Medicine and the pediatric intensive care units (PICUs) of Hacettepe University, School of Medicine in 2022. A total of 40 children with CHD (17 males, 23 females; mean age: 39.6±40.0 months; range, 2 to 216 months) who underwent open-heart surgery, while the control group consisted of 40 age- and sex-matched healthy children (18 males, 22 females; mean age: 42.8±46.6 months; range, 12 to 156 months) who attended the outpatient clinic and had no known chronic illness or medication usage. The patients with CHD were divided into two groups as cyanotic patients (n=18) and acyanotic patients (n=22). Patients with previously known chronic systemic conditions, such as renal failure, end-stage renal disease, dialysis requirements, hepatic failure, respiratory failure (including mechanical ventilation), local or systemic

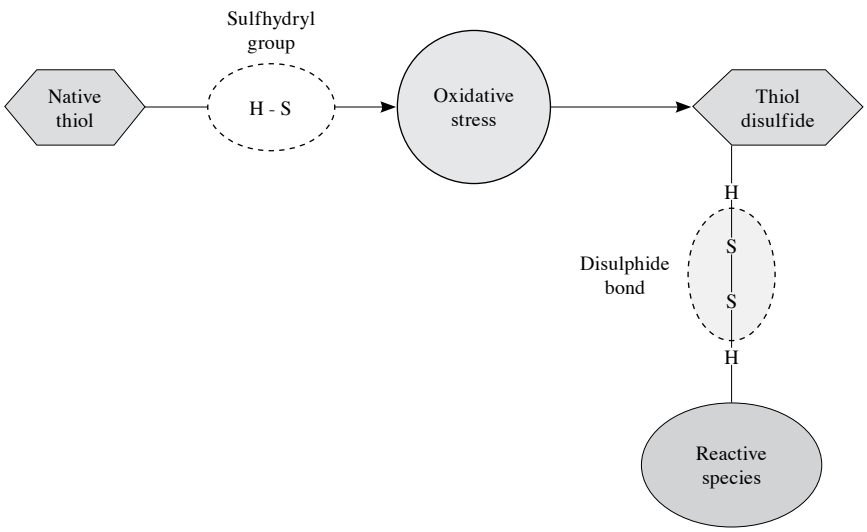

Figure 1. Thiol/disulfide homeostasis in oxidative stress.

infection, and inflammation were excluded from the study. Postoperative patients requiring extracorporeal membrane oxygenation (ECMO) were also excluded (Figure 2).

Biochemical parameters

After enrollment in the trial, all patients' complete blood count and biochemistry levels were examined. Venous blood specimens were collected from each participant to evaluate the TDH markers. For patients with CHD, blood samples were taken in the operating theater immediately before CPB (pre-CPB), immediately after CPB (post-CPB0), and 24 h after the surgical intervention (post-CPB24). The blood samples of the healthy volunteers were collected on admission at the outpatient clinic. Venous blood samples were collected in 5 mL tubes without gel (BD Vacutainer, NJ, USA) with a red cap to assess TDH parameters in serum. The samples were centrifuged at 3,000 rpm for 15 min to separate serum and plasma, and the serum samples were kept at –80ºC until all the samples were collected. Thiol/disulfide levels were measured by a new method defined by Erel and Neselioglu previously.[11,12] In this method, with measuring thiol levels (referred to as native thiol), disulfide levels were also assessed, and the combined sum of native thiol and disulfide levels was termed as total thiol.[11] After measuring the native thiol [SH], disulfide [SS] amounts, total thiol [SH+SS], and the disulfide/total thiol [SS/(SS+SH)], native thiol/total thiol [SH/(SH+SS)], and disulfide/native thiol [SS/SH] ratios were calculated. The TDH parameters were measured using an automated clinical chemistry analyzer (Cobas 501, Roche Diagnostics, Mannheim, Germany), and the results were reported in μmol/L. During the trial timeline, the clinical outcomes and patient information were concealed from all laboratory staff involved in analyzing plasma TDH measurements, including treating physicians, investigators, and study staff.

Perioperative variables

The procedures were classified according to the complexity of the surgery in line with the Risk Adjustment for Congenital Heart Surgery-1 (RACHS-1) scoring system.[13] Preoperative variables included age, sex, body mass index (BMI), and levels of albumin (mg/dL), and serum creatinine (Scr) (mg/dL) were

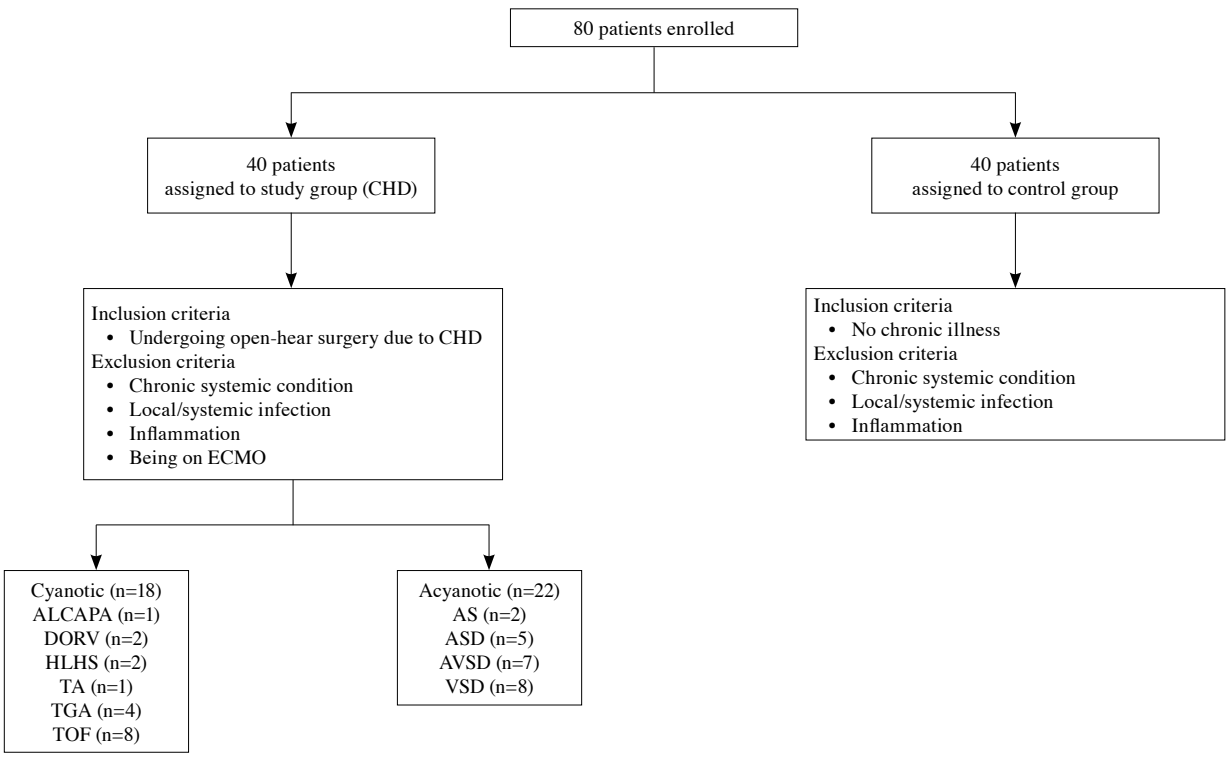

Figure 2. Study flowchart.

CHD: Congenital heart disease; ECMO: Extracorporeal membrane oxygenation; ALCAPA: Anomalous left coronary artery from the pulmonary artery; DORV: Double outlet right ventricle; HLHS: Hypoplastic left heart syndrome; TA: Truncus arteriosus; TGA: Transposition of great arteries; TOF: Tetralogy of Fallot; AS: Aortic stenosis; ASD: Atrial septal defect; AVSD: Atrioventricular septal defect; VSD: Ventricular septal defect.

recorded. The duration of CPB, aortic cross-clamp (ACC) time, and intraoperative fluid balance were recorded during the procedure. Postoperative variables recorded included the Pediatric Risk of Mortality-3 (PRISM-3), Pediatric Logistic Organ Dysfunction Score 2 (PELOD-2), maximum vasoactive inotropic score (VIS_{max}), Scr (mg/dL), postoperative fluid balance (mL/kg), urine output (mL/kg), mean lactate level (mmol/L), mixed venous saturation (%), and mechanical ventilation time (days).[14-16] Acute kidney injury (AKI) was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) classification system, with AKI categorized into Stages 1-3 based on changes in Scr and/or urine output.[17]

Statistical analysis

An *a priori* power analysis was conducted using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to determine the sample size required to test the study hypothesis. The sample size was calculated considering the medium or small effect size recommendation of Cohen.[18] The results indicated the required sample size to achieve 80% power for detecting medium effect (effect size= 0.65, α error 0.05, β error 0.20, distribution ratio to groups 1 [experimental and control]) was 39 patients for each group and a total of 78.

Statistical analysis was performed using the IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The Kolmogorov-Smirnov test was used to determine the distribution of numerical variables. Non-parametric methods were used for comparisons of native thiol, total thiol, and [SS/SH] levels, while parametric methods were used for comparisons of disulfide, [SS/(SH+SS)], and [SH/(SH+SS)] levels. The non-parametric Mann-Whitney U test and the parametric Student t-test were utilized to compare the variables between the study and control groups. The Mann-Whitney U test and independent sample t-test were used to compare two groups, while the Kruskal-Wallis test and analysis of variance (ANOVA) test were used to compare more than two groups. The relationship between categorical variables was analyzed using the chi-square test. Pre-CPB and post-CPB variables were compared using the paired t-test for parametric variables and Wilcoxon test for non-parametric variables. The Pearson correlation coefficient was calculated to establish a relationship between numerical variables with normal distribution, while the Spearman correlation coefficient was computed for non-normally distributed data. A *p* value of <0.05 was considered statistically significant.

RESULTS

Demographics and TDH parameters in CHD and control groups

Table 1 provides an overview of the patient and control groups' characteristic features and TDH

Table 1. Characteristics and the thiol/disulfide homeostasis of CHD patients and controls

	Control (n=40)			CHD (Pre-CPB) (n=40)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (month)			42.8±46.6			39.6±40.0	0.153[b]
Sex							0.822
Boy	18	45		17	42.5		
Girl	22	55		23	57.5		
Body mass index (kg/m^2)			15.1±2.44			14.62±2.50	0.124[a]
Albumin (mg/dL)			5.56±6.24			4.08±0.44	0.137[b]
SCr (mg/dL)			0.32±0.11			0.30±0.09	0.192[b]
Native thiol (µmol/L)			445.70±25.05			303.46±65.334	0.000*[b]
Total thiol (µmol/L)			480.84±28.76			331.52±72.86	0.000*[b]
Disulphide (µmol/L)			12.04±2.69			8.67±3.22	0.000*[a]
Disulphide/native(%)			3.93±0.98			4.71±4.29	0.028*[b]
Disulphid/total (%)			3.63±0.858			4.11±0.839	0.014*[a]
Native/total (%)			92.73±1.71			91.61±1.88	0.007*[a]

CHD: Congenital heart disease; Native: Native thiol; Pre-CPB: Values just before the cardiopulmonary bypass; SD: Standard deviation; Scr: Serum creatinine; Total: Total thiol; * Shows statistically significant (p<0.05); a: Independent sample t test; b: Mann Whitney U test.

parameters. The CHD and control groups were similar concerning age, sex, BMI, preoperative albumin and Scr levels (p>0.1). However, the mean native and total thiol levels were lower in CHD patients compared to the control group (native thiol: 303.46±65.334 *vs*. 445.70±25.05, p<0.0001; and total thiol: 331.52±72.86 *vs*. 480.84±28.76, respectively, p<0.0001), as well as a lower native thiol/total thiol ([SH]/[SH+SS]) ratio (p<0.01), compared to the control group (Table 1, Figure 3a). In contrast, the CHD group had higher [SS]/[SH] and [SS]/[(SH+SS)] ratios than the control group (p<0.05 and p<0.05, respectively) (Figure 4a).

TDH parameters in cyanotic and acyanotic CHD

The mean native and total thiol levels in the cyanotic patients were found to be significantly lower than those in the acyanotic patients and control group (native thiol: 287.11±73.90, 316.83±55.58, 445.70±25.05 p<0.0001; total thiol: 314.96±83.42, 345.07±61.63, 480.84±28.76, respectively, p<0.0001)

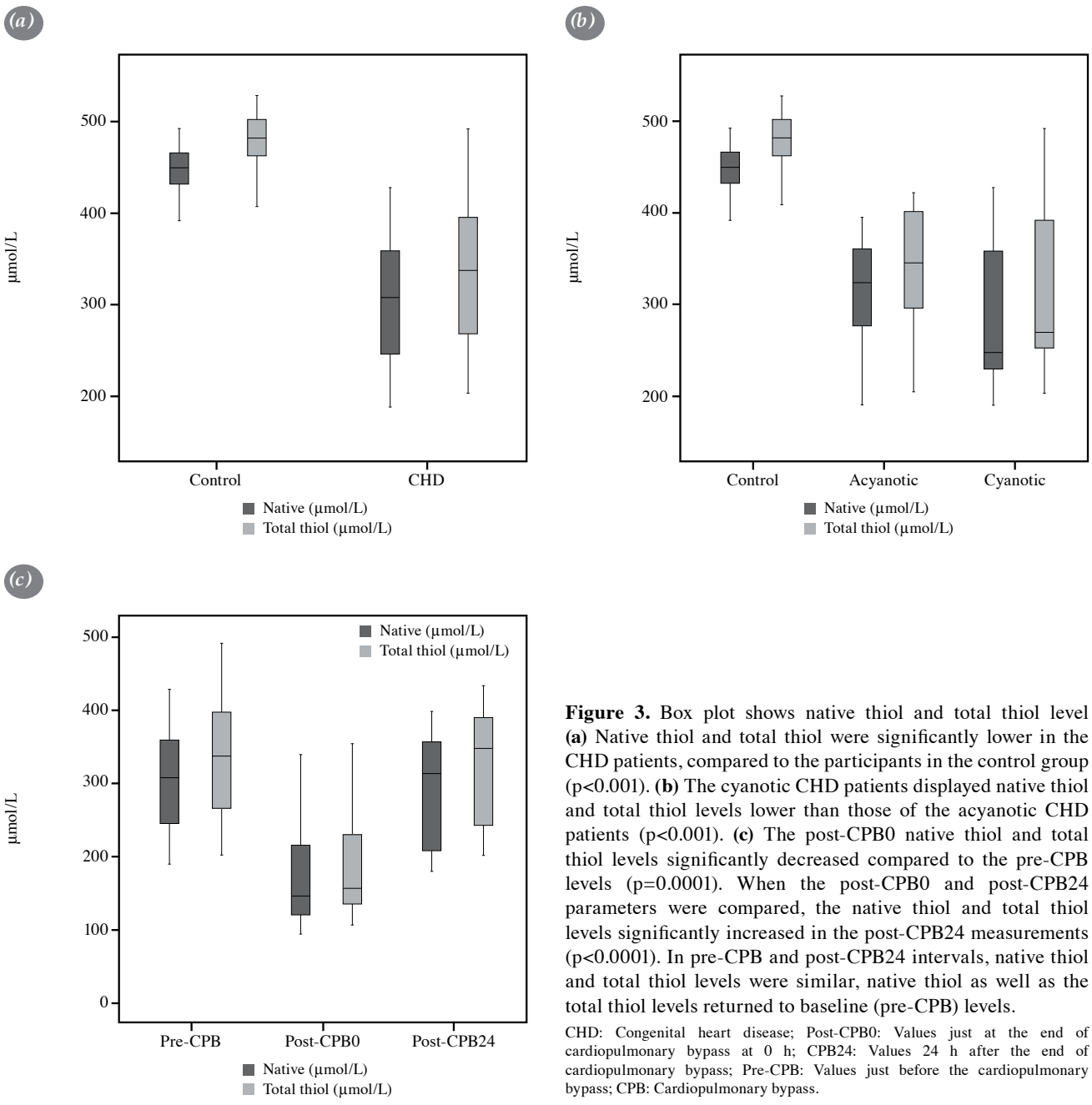

Figure 3. Box plot shows native thiol and total thiol level **(a)** Native thiol and total thiol were significantly lower in the CHD patients, compared to the participants in the control group (p<0.001). **(b)** The cyanotic CHD patients displayed native thiol and total thiol levels lower than those of the acyanotic CHD patients (p<0.001). **(c)** The post-CPB0 native thiol and total thiol levels significantly decreased compared to the pre-CPB levels (p=0.0001). When the post-CPB0 and post-CPB24 parameters were compared, the native thiol and total thiol levels significantly increased in the post-CPB24 measurements (p<0.0001). In pre-CPB and post-CPB24 intervals, native thiol and total thiol levels were similar, native thiol as well as the total thiol levels returned to baseline (pre-CPB) levels.

CHD: Congenital heart disease; Post-CPB0: Values just at the end of cardiopulmonary bypass at 0 h; CPB24: Values 24 h after the end of cardiopulmonary bypass; Pre-CPB: Values just before the cardiopulmonary bypass; CPB: Cardiopulmonary bypass.

(Figure 3b). Additionally, the cyanotic group exhibited higher mean disulfide levels (8.86±2.74 *vs*. 8.50±3.62, p<0.01) and higher ratios of [SS]/[SH] and [SS]/[SH+SS] compared to the acyanotic group (p<0.05 and p<0.05) (Figure 4b).

Table 2 summarizes the characteristics, clinical and laboratory features and TDH parameters of individuals with cyanotic and acyanotic CHD and the control group participants. Regarding the RACHS-1 score, the number of acyanotic patients scored as 1 and 2 were higher than the cyanotics patients (RACHS-1 score 1: 18.2% *vs*. 0, score 2: 68.2% *vs*. 5.6%, respectively, p<0.001). On the contrary, cyanotic patients being scored as 3 and 4 were statistically higher than acyanotic patients (RACHS-1 score 3: 66.7% *vs*. 13.6%, respectively, p<0.001). The mean

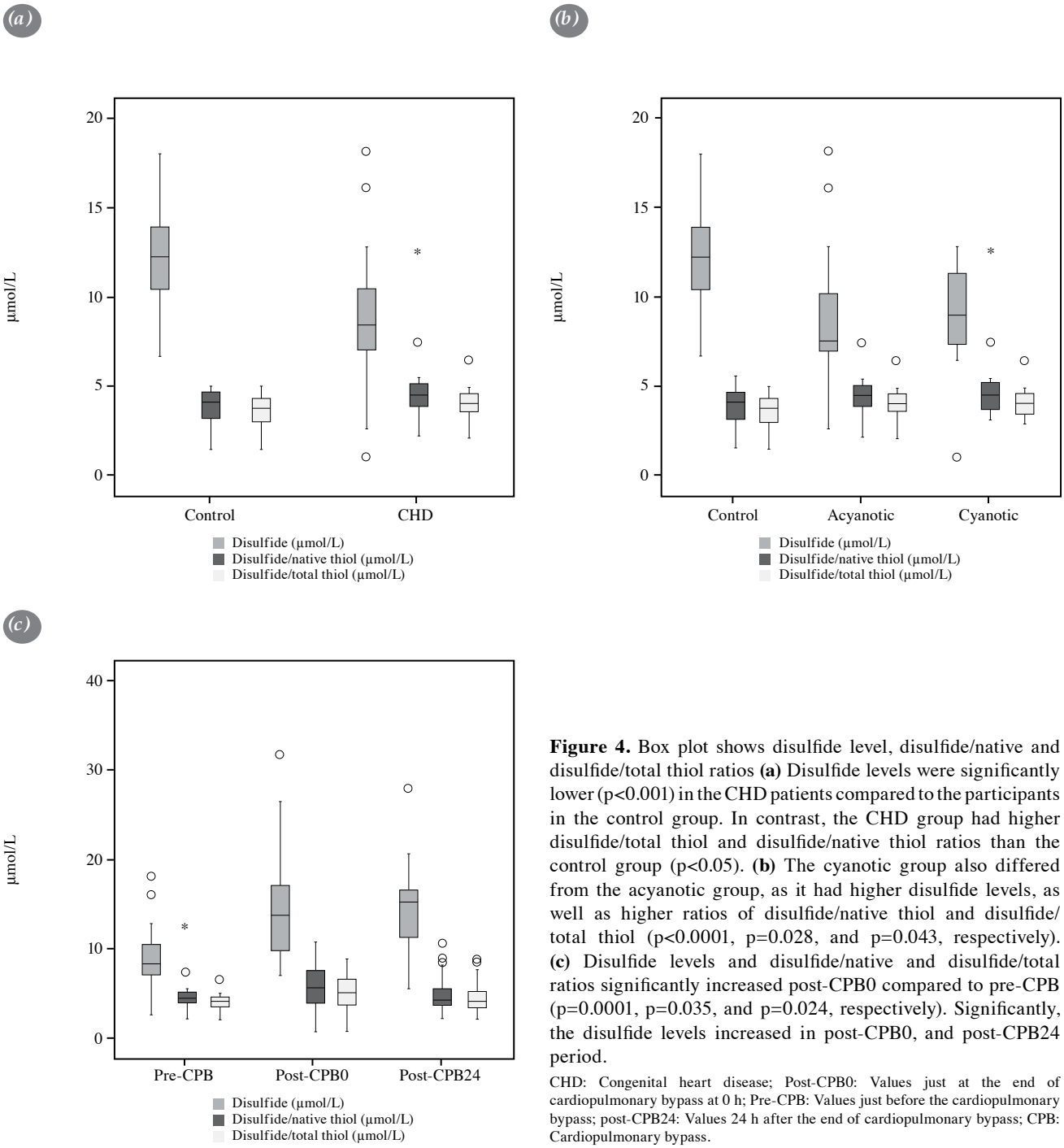

Figure 4. Box plot shows disulfide level, disulfide/native and disulfide/total thiol ratios **(a)** Disulfide levels were significantly lower (p<0.001) in the CHD patients compared to the participants in the control group. In contrast, the CHD group had higher disulfide/total thiol and disulfide/native thiol ratios than the control group (p<0.05). **(b)** The cyanotic group also differed from the acyanotic group, as it had higher disulfide levels, as well as higher ratios of disulfide/native thiol and disulfide/total thiol (p<0.0001, p=0.028, and p=0.043, respectively). **(c)** Disulfide levels and disulfide/native and disulfide/total ratios significantly increased post-CPB0 compared to pre-CPB (p=0.0001, p=0.035, and p=0.024, respectively). Significantly, the disulfide levels increased in post-CPB0, and post-CPB24 period.

CHD: Congenital heart disease; Post-CPB0: Values just at the end of cardiopulmonary bypass at 0 h; Pre-CPB: Values just before the cardiopulmonary bypass; post-CPB24: Values 24 h after the end of cardiopulmonary bypass; CPB: Cardiopulmonary bypass.

Table 2. Characteristics and the thiol/disulfide homeostasis of the cyanotic and acyanotic CHD patients and controls

	Acyanotic CHD (n=22)			Cyanotic CHD (n=18)			Control (n=40)			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (month)			42.50±48.22			36±33.50			42.8±46.6	0.165[b]
BMI (kg/m^2)			15.73±12.23			14.3±2.47			15.1±2.44	0.26
Sex										0.131
Boy	7	31.8		10	55.6		18	45		
Girl	15	68.2		8	44.4		22	55		
RACHS-1										0.000*
1	4	18.2		0	0		-	-		
2	15	68.2		1	5.6		-	-		
3	3	13.6		12	66.7		-	-		
4	0	0		4	22.2		-	-		
5	0	0		1	5.6		-	-		
PRISM-3			19.95±6.43			28.5±11.50			-	0.000*
PELOD-2			28.06±18.89			59.5±26.85			-	0.000*
$KDIGO_{max}$										0.036*
None	18	81		7	38.9		-	-		
1	0	0		1	5.6		-	-		
2	1	4.5		1	5.6		-	-		
3	3	13.6		9	50		-	-		
Fluid balance (mL)			117.18±82.45			263.38±200.94			-	0.009*[b]
Dialysis										0.000*
None	21	95.5		9	50		-	-		
Done	1	4.5		9	50		-	-		
CPB time (min)			57.95±23.50			105.73±52.90			-	0.000*[b]
ACC (min)			36.72±15.60			55.33±34.80			-	0.051[b]
MV (day)			1.27±2.07			15±22.16			-	0.000*[b]
VIS_{max}			0.72±0.82			1.83±.92			-	0.001*[b]
Lactate (µmol/L)			2.57±2.43			5.27±3.85			-	0.015*[b]
Mix venous (%)			61.27±12.15			52.38±19.98			-	0.383[b]
LOS in PICU (day)			5.00±5.18			19.66±21.72			-	0.001*[b]
LOS in hospital (day)			8.68±5.69			29.55±46.68			-	0.001*[b]
Mortality										0.001*
None	22	100		12	66.7		-			
Death	0	0		6	33.3		-			
Scr (mg/dL)			0.30±0.14			0.4±0.02			0.32±0.11	0.18[b]
Albumin (mg/dL)			4.22±0.411			3.89±0.43			5.56±6.24	0.137[b]
Native thiol (µmol/L)			316.83±55.58			287.11±73.90			445.70±25.05	0.000*[b]
Total thiol (µmol/L)			345.07±61.63			314.96±83.42			480.84±28.76	0.000*[b]
Disulphide (µmol/L)			8.50±3.62			8.86±2.74			12.04±2.69	0.01*[a]
Disulphide/native (%)			4.423±1.06			5.05±2.08			3.93±0.98	0.028*[b]
Disulphide/total (%)			4.05±0.89			4.18±0.78			3.63±0.858	0.043*[a]
Native/total (%)			91.89±1.78			91.27±1.99			92.73±1.71	0.071*[a]

CHD: Congenital heart disease; SD: Standard deviation; BMI: Body mass index; RACHS-1: Risk Adjustment for Congenital Heart Surgery-1; PRISM-3: Pediatric Risk of Mortality-3; PELOD-2: Pediatric Logistic Organ Dysfunction Score 2; KDIGO: Kidney Disease: Improving Global Outcomes; CPB: Cardiopulmonary bypass; ACC: Aortic cross-clamp; MV: Mechanical ventilation; VIS_{max}: Maximum vasoactive inotropic score; LOS: Length of hospital stay; Scr: Serum creatinine; Native: Native thiol; Total: Total thiol; * Shows statistically significant ($p<0.05$); a: One Way ANOVA test; b: Kruskal Wallis Test, Bonferroni correction for multiple comparisons.

PRISM-3 and PELOD-2 scores were higher in cyanotic patients compared to acyanotic patients (PRISM-3: 28.5±11.50 *vs*. 19.95±6.43, PELOD-2: 59.5±26.85 *vs*. 28.06±18.89, p<0.001). Regarding the development of AKI in cyanotic and acyanotic patients, 81% of the acyanotic patients did not develop any AKI. However, 50% of the cyanotic patients developed KDIGO Stage 3 AKI and required dialysis. Acute kidney injury and the need for dialysis were shown to be more prevalent in cyanotic patients (p<0.001). The mean CPB and ACC time were longer in cyanotic patients than acyanotic patients (CPB time: 105.73±52.90 *vs*. 57.95±23.50, respectively, p<0.001, ACC: 55.33±34.80 *vs*. 36.72±15.60, respectively, p<0.001). Mechanical ventilation duration, length of hospital stay (LOS) in PICU and LOS in the hospital were also found to be longer in cyanotic patients compared to the acyanotic patients (Table 2) (p<0.001).

Effect of CPB on TDH parameters

Table 3 compares the TDH parameters of pre-CPB, post-CPB0, and post-CPB24 in CHD patients. When pre- and post-CPB parameters were compared, the mean native thiol and total thiol levels significantly decreased in the post-CPB0 (native thiol: 303.46±65.334 to 170.45±66.73, p<0.0001; total thiol: 331.52±72.86 to 187.80±67.95, p<0.0001). Conversely, the post-CPB0 disulfide levels significantly increased compared to pre-CPB levels (p<0.05) (8.67±3.47 to 14.44±5.36; p<0.001). The mean post-CPB24 native and total thiol levels were elevated compared to post-CPB0 (native thiol: 170.45±66.73 to 293.46±72.3, p<0.0001; total thiol: 187.80±67.95 to 321.54±75.3, p<0.0001). However, no statistically significant differences were observed between pre-CPB and post-CPB24 (native thiol: 303.46±65.334 *vs*. 293.46±72.3, p=0.3; total thiol: 331.52±72.86 *vs*. 321.54±75.3, p=0.36). Comparing post-CPB0, and post-CPB24 disulfide levels significantly increased (14.44±5.36 to 15.34±3.86; p<0.001). Our results showed that CPB exposed patients to significant oxidative stress and decreased native and total thiol levels, whereas disulfide levels increased after CPB as an oxidative stress marker (Figures 3c and 4c).

Thiol-Disulfide homeostasis parameters and their relationship with mortality

In this study, we also compared TDH parameters between survivors and non-survivors (Table 4). Comparing post-CPB24 TDH parameters with pre-CPB parameters between survivors and deceased patients, most patients, except for the deceased ones, restored their antioxidant capacity and native thiol, as well as the total thiol levels returned to baseline (pre-CPB) levels. Comparing post-CPB0, and post-CPB24 disulfide levels between survivors and deceased patients, disulfide levels were significantly lower in deceased patients (post-CPB0: 14.44±5.36 *vs*. 10.53±1.05, p<0.05 and post-CPB24: 15.34±3.86 *vs*. 6.65±1.67, respectively, p<0.001), indicating that survivors responded better to oxidative stress than deceased patients.

Table 3. Comparison of parameters pre-CPB, immediately after CPB, and 24 h after CPB in CHD patient and control groups

	Control	CHD Pre-CPB (n=40)	CHD Post-CPB0 (n=40)			
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	t/Z	*p*
Native thiol (µmol/L)	445.70±25.05	303.46±65.334	170.45±66.73	293.46±72.3	5.48*	0.000[b]
Total thiol (µmol/L)	480.84±28.76	331.52±72.86	187.80±67.95	321.54±75.3	5.51*	0.000[b]
Disulphide (µmol/L)	12.04±2.69	8.67±3.22	13.86±5.14	14.04±4.78	4.95*	0.000[a]
Disulphide/native (%)	3.93±0.98	4.71±4.29	5.64±2.50	4.94±2.10	2.11*	0.035[b]
Disulphide/total (%)	3.63±0.858	4.11±0.839	4.98±2.02	4.47±1.68	2.34*	0.024[a]
Native/total (%)	92.73±1.71	91.61±1.88	90.02±4.04	91.05±3.38	2.20*	0.034[a]
Lactate (µmol/L)	-	1.12±0.72	3.57±2.29	1.53±1.21	4.08*	0.000[a]
Mix venous (%)	-	56.5±16.09	50.4±15.82	66.1±11.14	0.22*	0.8[a]

CPB: Cardiopulmonary bypass; CHD: Congenital heart disease; SD: Standard deviation; Native: Native thiol; Total: Total thiol; Pre-CPB: Values just before the cardiopulmonary bypass; Post-CPB0: Values just at the end of cardiopulmonary bypass at 0 h; Post-CPB24: Values 24 h after the end of cardiopulmonary bypass; t: Paired sample t test; Z: Wilcoxon test; * Value related comparison of Pre-CPB and Post-CPB0; a: Paired Sample test; b: Wilcoxon test.

Table 4. Relation of thiol/disulfide homeostasis parameters with mortality

	Control	CHD Pre-CPB (n=40)	CHD Post-CPB0 (n=40)	CHD Post-CPB24 (n=40)
	Mean±SD (μmol/L)	Mean±SD (μmol/L)	Mean±SD (μmol/L)	Mean±SD (μmol/L)
Native [mortality (-)] (n=34)	445.70±25.05	315.19±63.82	181.92±66.02	310.56±64.44
Native [mortality (+)] (n=6)	-	236.98±12.92	105.48±1.66	196.51±11.92
Z		-2.652	-3.447	-3.409
p		0.006[b]	0.000[b]	0.000[b]
Total thiol [mortality (-)] (n=34)	480.84±28.76	344.48±71.38	199.26±67.47	341.25±63.46
Total thiol [mortality (+)] (n=6)	-	258.05±12.67	122.81±3.68	209.83±10.34
Z		-2.652	-3.258	-3.864
p		0.00[b]	0.000[b]	0.000[b]
Disulphide [mortality (-)] (n=34)	12.04±2.69	8.67±3.47	14.44±5.36	15.34±3.86
Disulphide [mortality (+)] (n=6)	-	8.66±1.14	10.53±1.05	6.65±1.67
t		0.004	1.764	5.377
p		0.997[a]	0.046[a]	0.000[a]

CHD: Congenital heart disease; Pre-CPB: Values just before the cardiopulmonary bypass; Post-CPB0: Values just at the end of cardiopulmonary bypass at 0 h; Post-CPB24: Values 24 h after the end of cardiopulmonary bypass; SD: Standard deviation; Native: Native thiol; a: Independent sample t test; b: Mann-Whitney U test.

Correlation analysis between post-CPB24 TDH parameters and variables

A positive correlation was observed between post-CPB24 total and native thiol levels, mixed venous saturation, and albumin levels ($p<0.01$ and $p<0.05$, respectively). Conversely, a negative correlation was identified with CPB time, ACC time, lactate levels, LOS, PRISM-3, PELOD-2, and RACHS-1 scores ($p<0.05$) (Table 5).

DISCUSSION

In the present study, we compared the pre- and post-CPB TDH parameters in pediatric CHD patients. Post-CPB0 levels of native thiol and total thiol were significantly lower than pre-CPB levels. Our results indicated that CPB exposed patients to significant oxidative stress, resulting in a sharp decline in native and total thiol levels due to the consumption of antioxidant capacity. However, disulfide levels increased after CPB as an oxidative stress marker and continued to rise with the maximum level measured in the post-CPB24 period. This indicates that the oxidative stress response initiates during CPB and persists for 24 h, resulting in a shift in TDH balance toward disulfide formation due to thiol oxidation. These findings are in line with the study by Sanrı et al.,[18] in which they examined TDH parameters in patients undergoing on-pump coronary artery bypass grafting at three distinct time points: pre-ischemia, ischemia, and post-ischemia periods. During the ischemia period, thiol levels decreased while disulfide levels increased, showing a significant correlation between disulfide levels and ACC time. By the end of the ischemia, both thiol and disulfide levels increased.

The current study showed that individuals with CHD had significantly lower levels of native thiol and total thiol than the control group ($p<0.001$). It indicates that their antioxidant reserve capacity is lower than the healthy population. Our results are consistent with the previous studies. Farías et al.[19] showed that CHD patients were constantly exposed to oxidative stress due to cardiac and respiratory problems from early infancy, leading to a lower antioxidant capacity than healthy children. Rokicki et al.,[20] examining the antioxidant capacity of CHD patients, found higher oxidative markers and lower antioxidant capacity than the normal population. In our study, we classified the CHD patients into cyanotic and acyanotic groups, and we found that native thiol and total thiol levels were

Table 5. Correlation analysis between post-CPB24 thiol/disulfide homeostasis parameters and variables

Spearman's rho/Pearson (r)		Post-CPB24 (SH)	Post-CPB24 (SH) + (SS)	Post-CPB24 (SS)	CPB	Aortic clamp	LAC	Mix	LOS	PRISM3	PELOD2	RACHS	Alb24
Post-CPB24 [SH]	rho/r	1.000	.987**	0.561**	-0.461**	-0.438**	-0.319*	0.428**	-0.444**	-0.446**	-0.407**	-0.532**	0.392*
	p		0.000	0.000	0.003	0.005	0.045	0.006	0.004	0.004	0.009	0.000	0.012
Post-CPB24 [SH] + [SS]	rho/r		1.000	0.523**	-.469**	-0.459**	-0.343*	0.421**	-0.445**	-0.451**	-0.401*	-0.528**	0.382*
	p			0.001	0.002	0.003	0.030	0.007	0.004	0.003	0.010	0.000	0.015
Post-CPB24 [SS]	rho/r			1.000	-0.319*	-0.365*	-0.277	0.465**	-0.438**	-0.306	-0.291	-0.319*	0.341*
	p				0.045	0.021	0.083	0.003	0.005	0.055	0.068	0.045	0.031

CPB: Cardiopulmonary bypass; Post-CPB24: Values 24 h after the end of cardiopulmonary bypass; SH: Native thiol; SH: Total thiol; SS: Disulfide; LAC: Lactate; Mix: Mix venous saturation; LOS: Length of hospital stay; PRISM: Pediatric risk of mortality score; PELOD: Pediatric logistic organ dysfunction; RACHS: Risk adjustment of congenital heart surgery; * Correlation is significant at 0.05; ** Correlation is significant at 0.01.

lower in cyanotic patients than in acyanotic patients. In contrast, disulfide levels were higher in cyanotic patients than in acyanotic patients. In a previous study, similar results showed that native thiol and total thiol levels were lower in CHD patients than in healthy individuals and disulfide levels were markedly higher in cyanotic patients than in acyanotic patients.[21] Clinical outcomes for children with cyanotic CHD are known to be worse than those with acyanotic CHD. In our study, cyanotic CHD patients had also worse peri- and postoperative outcomes than acyanotic patients. A potential reason for this may be that patients with cyanotic CHD are more difficult to treat and at greater surgical risk, as scored with the RACHS-1 score. Another important reason may be cyanotic patients' lower antioxidant capacity than acyanotic patients. It is evident that cyanotic patients experience hypoxia, ischemia-reperfusion injury, and oxidative stress more frequently than acyanotic patients from early infancy. Additionally, compared to acyanotic patients, cyanotic patients have considerably higher levels of oxidative markers.[22,23] Temel et al.[21] demonstrated that TDH parameters served as indicators of oxidative stress and exhibited distinct characteristics among cyanotic and acyanotic CHD patients and controls. Sogut et al.[24] showed similar results to our study. They found that native and total thiol levels were lower in cyanotic patients compared to acyanotic patients. In contrast, disulfide levels were higher in cyanotic patients in their study.[24] They also compared pre- and postoperative TDH parameters. However, they did not investigate the relationship between the prognosis or outcomes of CHD patients and the pre- and postoperative TDH parameters. In our study, we compared TDH parameters before CPB between deceased and surviving patients. We found that native and total thiol levels were lower in deceased patients, with the lowest levels observed during the post-CPB0 period. Among the survivors, there was no statistically significant difference in native and total thiol parameters between pre-CPB and post-CPB24, indicating that these values returned to their baseline levels (pre-CPB) within the initial 24 h after CPB. However, post-CPB24 parameters of native and total thiol levels showed a marked decrease in deceased patients compared to pre-CPB levels, indicating their inability to restore these levels during the post-CPB24 period. In the postoperative period, disulfide thiol levels were notably higher in the survivors than in the deceased patients, underscoring the superior response of survivors to oxidative stress compared to the deceased patients. This finding suggests that TDH parameters can serve as predictors of mortality.

In the current study, both antioxidant capacity and antioxidant response and the ability to maintain this response throughout the period of exposure to oxidative stress significantly affected mortality and morbidity. Patients with low baseline antioxidant capacity undergoing additional procedures, such as CPB and ACC, that increase the oxidative stress load and who cannot create an adequate oxidative response may experience life-threatening consequences. The longer the ACC and CPB time, the higher the risk of oxidative stress and related complications.[25] In our study, we found a negative correlation between post-CPB24 native thiol and total thiol levels versus ACC and CPB time (r=-0.438 and r=-0.459; r=-0.461, and r=-0.469, respectively, $p<0.01$).

It is important to highlight that CPB and oxidative stress can trigger inflammation and severe endothelial injury. This, in turn, leads to dysfunction in endothelial progenitor cells, a cytokine storm, and systemic inflammatory response syndrome.[26-28] Subsequently, this cascade prompts the formation of microthrombi and platelet aggregation, which hampers organ perfusion. This progression ultimately reduces cardiac contractility and culminates in low cardiac output syndrome. The TDH parameters hold significant potential to serve as early and reliable biomarkers, functioning as prognostic indicators in the future. Given their impact on mortality, the early identification of impaired TDH could pave the way for timely interventions, including plasma exchange, continuous hemodiafiltration, vasoactive agents and antioxidants, and immunomodulation. By evaluating TDH parameters and their derivatives, assessing patients' preoperative antioxidant capacity is feasible. This, in turn, permits the implementation of scheduled antioxidant treatments (e.g., vitamin C, thiol-containing antioxidants such as N-acetylcysteine, glutathione, and taurine) in elective surgeries. This proactive approach aims to enhance antioxidant capacity leading to the surgical procedure.[29-31] Evaluating patients' antioxidant capacities and oxidative responses by TDH parameters during the intra- and postoperative period of intensive care follow-ups may predict morbidity and mortality. Standardized use of TDH parameters requires more clinical studies to reach a consensus, and we believe that the current study can pioneer further conducted studies.

The main limitation to this study is its small sample size. Having data from only two different centers, the results of this study cannot be generalized for the entire CHD population. The second limitation is studying TDH with well-known oxidative stress markers, such as total oxidant status, total antioxidant status, and malondialdehyde, which may better reveal its value as an oxidative stress marker in CHD patients.[32] Another limitation is that we only evaluated pre-CPB and post-CPB0, and post-CPB24 thiol levels. Thiol parameters before, during, and after dialysis were not evaluated for patients who required dialysis, nor were thiol levels evaluated before, during, and after AKI for AKI patients. Additionally, we excluded patients who required ECMO, since our study focused only on the oxidative stress effect of CPB. However, ECMO itself is known to contribute to oxidative stress. Future studies should evaluate the effect of ECMO on thiol/disulfide parameters.

In conclusion, our study results suggest that thiol/disulfide measurements hold promising evidence for their use as a biomarker in determining the pre- and post-cardiopulmonary bypass oxidative status of patients undergoing congenital heart disease surgery. Moreover, the interpretation of thiol/disulfide levels has emerged as a candidate biomarker for predicting the outcomes of these patients.

Ethics Committee Approval: The study protocol was approved by the Koç University Ethics Committee (date: 21.10.2022, no: 2022.306.IRB2.053). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from the parents and/or legal guardians of the patients.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept: M.T., B.B., O.E.; Design: M.T., B.B., O.E.; Supervision: M.T., O.E.; Materials: M.T., U.K., M.U.Y, S.O., M.B.; Data Collection and/or Processing: M.T., A.F.Y., M.A., M.U.Y., S.O., U.K., M.B.; Analysis and/or interpretation: M.T., M.A., M.B.; Literature review: M.T., U.K., M.U.Y.; References and funding: Writing: M.T., S.O., M.A., A.F.Y., Critical review: M.T., B.B., A.F.Y.

Conflict of Interest: 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. Data and Statistics on Congenital Heart Defects CDC. Available at: https://www.cdc.gov/ncbddd/heartdefects/data.html. [Accessed: 10.06.2023]

2. McDonald CI, Fraser JF, Coombes JS, Fung YL. Oxidative stress during extracorporeal circulation. Eur J Cardiothorac Surg 2014;46:937-43. doi: 10.1093/ejcts/ezt637.
3. Madhavan S, Chan SP, Tan WC, Eng J, Li B, Luo HD, et al. Cardiopulmonary bypass time: Every minute counts. J Cardiovasc Surg (Torino) 2018;59:274-81. doi: 10.23736/S0021-9509.17.09864-0.
4. Ellgaard L, Sevier CS, Bulleid NJ. How are proteins reduced in the endoplasmic reticulum? Trends Biochem Sci 2018;43:32-43. doi: 10.1016/j.tibs.2017.10.006.
5. Yazar H, Kayacan Y, Erel O. Thiol-disulfide homeostasis as an oxidative stress indicator: Applications to nutrition. In book: Biomarkers in Nutrition 2022:801-18. doi: 10.1007/978-3-031-07389-2_47.
6. Kaman A, Aydın Teke T, Gayretli Aydın ZG, Karacan Küçükali G, Neşelioğlu S, Erel Ö, et al. Dynamic thiol/disulphide homeostasis and pathogenesis of Kawasaki disease. Pediatr Int 2019;61:913-8. doi: 10.1111/ped.13958.
7. Kundi H, Ates I, Kiziltunc E, Cetin M, Cicekcioglu H, Neselioglu S, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med 2015;33:1567-71. doi: 10.1016/j.ajem.2015.06.016.
8. Otal Y, Kahraman FA, Haydar FG, Erel Ö. Dynamic thiol/disulfide homeostasis as oxidative stress marker in diabetic ketoacidosis. Turk J Med Sci 2021;51:743-8. doi: 10.3906/sag-1904-55.
9. Aydogan S, Akduman H, Dilli D, Koyuncu E, Çitli R, Erel Ö, et al. The role of thiol-disulfide homeostasis in neonatal sepsis. J Matern Fetal Neonatal Med 2021;34:1522-8. doi: 10.1080/14767058.2019.1638904.
10. Harris C, Hansen JM. Oxidative stress, thiols, and redox profiles. Methods Mol Biol 2012;889:325-46. doi: 10.1007/978-1-61779-867-2_21.
11. Erel Ö, Erdoğan S. Thiol-disulfide homeostasis: An integrated approach with biochemical and clinical aspects. Turk J Med Sci 2020;50:1728-38. doi: 10.3906/sag-2003-64.
12. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32. doi: 10.1016/j.clinbiochem.2014.09.026.
13. Jenkins KJ, Gauvreau K, Newburger JW, Spray TL, Moller JH, Iezzoni LI. Consensus-based method for risk adjustment for surgery for congenital heart disease. J Thorac Cardiovasc Surg 2002;123:110-8. doi: 10.1067/mtc.2002.119064.
14. Pollack MM, Patel KM, Ruttimann UE. PRISM III: An updated Pediatric Risk of Mortality score. Crit Care Med 1996;24:743-52. doi: 10.1097/00003246-199605000-00004.
15. Leteurtre S, Duhamel A, Salleron J, Grandbastien B, Lacroix J, Leclerc F; Groupe Francophone de Réanimation et d'Urgences Pédiatriques (GFRUP). PELOD-2: An update of the PEdiatric logistic organ dysfunction score. Crit Care Med 2013;41:1761-73. doi: 10.1097/CCM.0b013e31828a2bbd.
16. Gaies MG, Gurney JG, Yen AH, Napoli ML, Gajarski RJ, Ohye RG, et al. Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. Pediatr Crit Care Med 2010;11:234-8. doi: 10.1097/PCC.0b013e3181b806fc.
17. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Inter Suppl; 2012;2:1-138. Available at: https://kdigo.org/wp-content/uploads/2016/10/KDIGO-2012-AKI-Guideline-English.pdf [Accessed: 10.06.2023]
18. Sanrı US, Özsin KK, Toktaş F, Balcı AB, Üstündağ Y, Huysal K, et al. The effect of thiol-disulfide homeostasis in patients undergoing on-pump coronary artery bypass grafting. Turk Gogus Kalp Dama 2019;27:484-92. doi: 10.5606/tgkdc.dergisi.2019.18033.
19. Farías JG, Molina VM, Carrasco RA, Zepeda AB, Figueroa E, Letelier P, et al. Antioxidant therapeutic strategies for cardiovascular conditions associated with oxidative stress. Nutrients 2017;9:966. doi: 10.3390/nu9090966.
20. 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.
21. Temel MT, Demiryürek S, Saracaloglu A, Ozer CH, Kahraman HN, Coskun ME, et al. Determination of dynamic thiol/disulphide homeostasis in children with tetralogy of Fallot and ventricular septal defect. Cardiol Young 2019;29:499-504. doi: 10.1017/S104795111900012X.
22. 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.
23. Pirinccioglu 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. doi: 10.1111/j.1442-200X.2011.03478.x.
24. Sogut I, Kar F, Senat A, Duymaz T, Erel O, Salihoglu E. et al. Thiol/disulfide homeostasis and oxidant status in children with congenital heart disease. Turk Biyokim Derg 2022;47:257-65. doi: 15/tjb-2021-0172.
25. Gil-Gómez R, Blasco-Alonso J, Castillo-Martín R, Milano-Manso G. Prognostic indicators after cardiac surgery in children and their relationship with the oxidative stress response. Rev Esp Anestesiol Reanim 2016;63:3-12. Spanish. doi: 10.1016/j.redar.2015.01.015.
26. Satriano A, Franchini S, Lapergola G, Pluchinotta F, Anastasia L, Baryshnikova E, et al. Glutathione blood concentrations: A biomarker of oxidative damage protection during cardiopulmonary bypass in children. Diagnostics (Basel) 2019;9:118. doi: 10.3390/diagnostics9030118.
27. McDonald CI, Fraser JF, Coombes JS, Fung YL. Oxidative stress during extracorporeal circulation. Eur J Cardiothorac Surg 2014;46:937-43. doi: 10.1093/ejcts/ezt637.
28. Millar JE, Fanning JP, McDonald CI, McAuley DF, Fraser JF. The inflammatory response to extracorporeal membrane oxygenation (ECMO): A review of the pathophysiology. Crit Care 2016;20:387. doi: 10.1186/s13054-016-1570-4.
29. Reiff DD, Cron RQ. Cytokine storm syndrome triggered by extracorporeal membrane oxygenation in pediatric patients. Children (Basel) 2023;10:1052. doi: 10.3390/children10061052.
30. Hill A, Borgs C, Fitzner C, Stoppe C. Perioperative vitamin C and E levels in cardiac surgery patients and their clinical significance. Nutrients 2019;11:2157. doi: 10.3390/nu11092157.

31. Sucu N, Cinel I, Unlu A, Aytacoglu B, Tamer L, Kocak Z, et al. N-acetylcysteine for preventing pump-induced oxidoinflammatory response during cardiopulmonary bypass. Surg Today 2004;34:237-42. doi: 10.1007/s00595-003-2699-8.

32. Temel HH, Kumbasar U, Büber E, Aksoy Y, Cavdar S, Dogan R, et al. Comparison of antioxidant reserve capacity of children with acyanotic & cyanotic congenital heart disease. Indian J Med Res 2020;152:626-32. doi: 10.4103/ijmr.IJMR_2215_18.