Research Article

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Thiol/disulfide homeostasis and oxidant status in children with congenital heart disease

Doğumsal kalp hastalığı olan çocukların tiyol/disülfid dengesi ve oksidatif durumu

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Abstract

Objectives: This article aims to explain the altered oxidative status and thiol/disulfide homeostasis before and after surgery in children with congenital heart disease (CHD).
Methods: Blood samples were taken from the patients (n=50) before the operation (baseline), at the 1st hour, and at the 24th hour after the operation. Thiol-disulfide levels, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), myeloperoxidase (MPO), ceruloplasmin, albumin, ischemia-modified albumin (IMA), and prolidase activities of all samples were measured. Preoperative oxygen saturation (SaO_2) values and neutrophil/lymphocyte ratios (NLR) were also measured.
Results: Before the operation, TOS, OSI, MPO, ceruloplasmin, IMA, NRL, and disulfide levels were higher in the cyanotic group than in the acyanotic group. When the indicated three different time points were compared, the TOS, OSI levels, and MPO activities of the 24th hour were significantly lower than the baseline and 1st hour. In comparison, ceruloplasmin levels of 1st hour were significantly higher than of the baseline and 24th hour. Native thiol and total thiol levels in the baseline group were significantly lower than in the 1st and 24th hours. Disulfide levels of the 24th hour were significantly lower than of the baseline.
Conclusions: The operation leads to changes in the thiol-disulfide balance and oxidant status in CHD.

Keywords: ceruloplasmin; congenital heart surgery; disulfide; ischemia modified albumin; myeloperoxidase (MPO); oxidative stress; prolidase; thiol.

Öz

Amaç: Bu makale konjenital kalp hastalığı (KKH) olan çocuklarda operasyon öncesi ve sonrası değişen oksidatif durum ve tiyol/disülfid homeostazını açıklamayı amaçlamaktadır.
Yöntem: Hastalardan (n=50) operasyon öncesi (başlangıç), operasyon sonrası 1. saat ve 24. saatte kan örnekleri alındı. Bütün örneklerin tiyol-disulfid seviyeleri, total antioksidan durum (TAS), total oksidan durum (TOS), oksidatif stres indeksi (OSI), miyeloperoksidaz (MPO), seruloplazmin, albumin, iskemik modifiye albumin (IMA)

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ve prolidaz aktivitesi ölçüldü. Bunun yanı sıra operasyon öncesi oksijen saturasyon (SaO_2) değeri ve nötrofil/lenfosit oranı (NLR) ölçüldü.

Bulgular: Ameliyat öncesi siyanotik grubun TOS, OSI, MPO, seruloplazmin, IMA, NRL ve disülfid düzeyleri asiyanotik gruba göre daha yüksekti. Üç farklı zaman noktasını birbiriyle karşılaştırdığımızda 24. saatteki TOS, OSI seviyeleri ve MPO aktiviteleri başlangıç ve 1. saate göre anlamlı derecede düşük, 1. saat seruloplazmin seviyeleri ise başlangıç ve 24. saate göre anlamlı derecede yüksekti. Başlangıç grubunda doğal tiyol ve toplam tiyol düzeyleri 1. ve 24. saate göre anlamlı derecede düşüktü. 24. saatteki disülfid seviyeleri başlangıca göre önemli ölçüde düşüktü.

Sonuç: Ameliyat, KKH'li çocuklarda tiyol-disülfid dengesinde ve oksidan durumunda değişikliklere yol açar.

Anahtar sözcükler: disülfid; Doğuşsal kalp cerrasi; iskemik modifiye albumin; miyeloperoksidaz; oksidatif stres; prolidaz; seruloplazmin; tiyol.

Introduction

Congenital heart disease (CHD) includes hereditary structural or functional anomalies in the cardiovascular system that can be identified at birth or later. CHD is the prominent reason for pediatric deaths in industrialized nations [1]. CHD covers more than one problem that affects the healthy function of the heart. Stress that occurs during or after surgery for CHD is one of these problems and triggers biochemical reactions [2]. In some cases, cytokines and other factors involved in inflammatory processes may be overactive by disrupting the homeostasis and causing severe damage during or after surgery [3]. Therefore, in open-heart surgery, children require more precision and care than adults. Although there are a few studies on the oxidative stress state of children after cardiac surgery, there are no studies on thiol/disulfide equilibrium [4]. Studies investigating surgical outcomes in children with CHD have shown that pre-operative data (parameters such as age, body surface area, oxygen saturation, neutrophil ratio, leukocyte count, CRP, albumin level) influence clinical outcomes [5].

Oxidative stress studies are frequently highlighted CHD mechanisms due to the impairment of molecular and cellular functions and the loss of balance between the assembly of free radicals or reactive oxygen species (ROS) and, therefore, the inhibitor system. ROS are primary molecules that cause oxidative damage above physiological levels. Enzymatic or non-enzymatic defense mechanisms guard the organism from the harmful effects of ROS [6]. Thiol, one of all the non-enzymatic antioxidants, is a compound containing sulfhydryl (–SH) cluster that plays a vital role in preventing the formation of oxidative stress state in cells. Thiol units are oxidized by ROS and regenerate into reversible disulfide bonds. The disulfide bond structures may be reduced again back to thiol units, keeping the thiol-disulfide in equilibrium. Thiol/disulfide balance plays a vital role in antioxidant defense, detoxification, apoptosis, accelerator activities, transcription, and cellular signal pathway [7].

This is the first study in which thiol/disulfide homeostasis and oxidant status are evaluated together before and after surgery. Our hypothesis is that oxidative stress is increased pre-operatively due to CHD and post-operatively due to open-heart surgery. Depending on the stress, the level of disulfide increases, and the level of thiol decreases. After the reduction of the stress conditions, both the oxidant and thiol/disulfide status are balanced. For this purpose, changes in thiol/disulfide balance and oxidative stress parameters were evaluated according to pre-operative cyanotic and acyanotic status in pediatric patients with CHD who underwent open-heart surgery. Afterward, the changes in thiol/disulfide balance and oxidative stress parameters before and after surgery were investigated in pediatric patients with CHD who underwent open-heart surgery.

Materials and methods

Patients

Fifty consecutive pediatric patients who underwent open-heart surgery because of CHD were examined in this study. The same operative team performed all operations. The patients were diagnosed with tetralogy of Fallot (n=15), transposition of the great arteries (n=6), truncus arteriosus (n=6), total anomalous pulmonary venous drainage (n=3), atrial septal defect (n=3), ventricular septal defect (n=10), atrioventricular septal defect (n=1), patent ductus arteriosus (n=1), aortic stenosis (n=2), and coarctation of the aorta (n=3). In the study groups, the gender distribution was 25 females and 25 males (n=50). The mean age of the study group was 5.4 ± 2.92, and their ages ranged from one week to 13 years. Patients with a history of pre-operative infection, blood product transfusion, inotropic support, oxygen supply in the last two weeks, and patients who need extracorporeal membrane oxygenation (ECMO) support post-operatively were excluded from the study. All procedures were conducted under general anesthesia with mild hypothermic cardiopulmonary bypass. Intermittent isothermic blood cardioplegia was used for myocardial protection. General anesthesia was maintained with sevoflurane, and intravenous rocuronium was used for neuromuscular blocking. Blood samples of these patients were taken into biochemistry tubes before surgery and after the 1st hour and 24th hours of surgery. Their serum was obtained (centrifuged at 1500×*g* at 4 °C for 10 min) and stored at –80 °C in the

biochemistry laboratory. Initially, the patients were divided into two groups according to their preop-oxygen saturation (SaO_2) values (Radiometer ABL 800, Bronshoj, Denmark). Thirty of them were cyanotic, and 20 were acyanotic. Finally, blood samples taken from the patients at three different time points were divided into groups (baseline; pre-operative, 1st hour; post-operative 1st hour and 24th hour; 24th postoperative hour) and then thiol-disulfide homeostasis, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), myeloperoxidase (MPO), ceruloplasmin activities, albumin level, ischemia modified albumin (IMA) level, and prolidase activities were studied in all groups with a fully automated system (Siemens Advia 1800, Erlangen, Germany). In addition, neutrophil/lymphocyte ratios (NLR) and albumin were also measured (Siemens Advia 2120i, Erlangen, Germany). The study group included patients who did not have metabolic disorders and chronic liver and kidney disease. The local clinical research ethics committee of the university approved the study protocol (2021-40034-12).

Biochemical analyzes

Oxidant and antioxidant status: Commercial kits were used to assess the TAS (mmol Trolox eq/L), TOS (mol H_2O_2 eq/L), and OSI (arbitrary unit) (Rel Assay Diagnostics, Gaziantep, Turkey) [7]. With antioxidants, a dark blue-green 2, 2′-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical is reduced to a colorless reduced ABTS form. The change in absorbance at 660 nm is proportional to the TAS level of the sample. The ferrous ion-chelator complex is oxidized to ferric ion by oxidants in the serum sample. In an acidic media, the ferric ion forms a colorful complex with xylenol orange. The change in absorbance at 530 nm is proportional to the sample's TOS level. The OSI levels in the serum sample were determined by dividing the TOS level by the TAS level.

Myeloperoxidase (MPO) activity: MPO activity was detected by measuring the rate of product formation from the oxidation of o-dianisidine with MPO activity in the presence of hydrogen peroxide (H_2O_2) at 460 nm using a kinetic calculation [8]. One unit of MPO was defined as one mol of H_2O_2 degraded per minute at 25 °C, and U/L of serum was used to represent MPO activity.

Ceruloplasmin activity (ferroxidase activity): Serum ceruloplasmin activities (U/L) were determined using Sitto's process [9]. In order to determine the ferroxidase activity in the sample, firstly, acetate buffer is added to o-dianisidine as a substrate that the ferroxidase enzyme can oxidize. The increase in absorbance correlates with the increased oxidized substrate with enzyme activity. Absorbance monitoring was performed at 445 nm.

Ischemia-modified albumin level (IMA): Das et al. published a method for measuring ischemia-modified albumin level (IMA-ABSU) in serum [10]. The color production of albumin-cobalt with dithiothreitol (DTT) reaction was calculated with spectroscopy using this method (470 nm).

Prolidase activity: The activity of serum prolidase was measured using the method defined by Myara et al. [11]. Briefly, 100 µL of serum is mixed with 100 µL of serum physiological solution. At 37 °C for 30 min, a 75 µL pre-incubation mixture was combined with a 25 µL reaction mixture. Next, 100 µL of 144 mmol/L gly-pro (pH 7.8) solution was applied to this mixture and incubated at 37 °C for 5 min. After that, 1 mL of glacial acetic acid was added to stop the reaction, and the mixture was incubated for an additional 5 min at 37 °C. One milliliter of glacial acetic acid was added to finish the incubation reaction. Then 300 µL Tris HCl buffer solution (pH 7.8) and 1 mL ninhydrin reagent (3 g/dL ninhydrin dissolved in 0.5 mol/L orthophosphoric acids) were added and incubated for 20 min at 90 °C. The absorbance at 515 nm was used to assess the proline amount.

Thiol-disulfide homeostasis measurement: Erel and Neselioglu used the automated spectrophotometric method to determine the thiol-disulfide homeostasis tests in the serum [12]. This method is based on the detection of functional –SH groups with 5′-5′-dithiobis-(-2-nitrobenzoic) acid (DTNB) and the reduction of the dynamic disulfide bond (–S–S) with sodium borohydride ($NaBH_4$). The thiol content of the samples was determined using DTNB as a thiol chromogen. The DTNB molecules were transformed to 5-thionitro-benzoic acid by free SH- groups, and molecular density was determined at 412 nm 2-mercaptoethanol was used as a calibrator for the assay. The disulfide levels in the samples were determined by subtracting the native thiol levels from the total thiol levels and dividing them by two, followed by the disulfide/native thiol percent ratios, disulfide/total thiol percent ratios, and native thiol/total thiol percent ratios.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 22.0 software was used for statistical analysis (IBM Corp.; Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normality of distribution. The analysis of variance (ANOVA) test using the post-hoc Tukey test was applied for data that showed compliance with the normal distribution, while for data for which the normal distribution did not fit, the Kruskal–Wallis test with the post-hoc Dunn's multiple comparison test was applied. The student's t-test (for the parametric variables) and the Mann–Whitney U test (for the non-parametric variables) were performed to pairwise comparison. Normally distributed data were expressed as the mean ± standard deviation, and the data that were not normally distributed were expressed as the median (interquartile range (IQR); 25th–75th percentile), $p<0.05$ was considered significant. Pearson correlation analysis was performed when the variables were suitable for the normal distribution, and Spearman correlation analysis when they were not suitable for the normal distribution, $p<0.05$ was considered significant.

Results

Variables for both the cyanotic and acyanotic groups in the pre-operation (preop)

When the patients were evaluated according to preoperative cyanotic and acyanotic status, TOS, OSI, MPO, ceruloplasmin, and IMA levels of cyanotic patients were higher than in acyanotic patients ($p<0.05$, $p<0.01$, $p<0.05$, $p<0.05$, and $p<0.05$, respectively). TAS, albumin levels, and prolidase activities of cyanotic patients were lower than in

acyanotic patients (p<0.01, p<0.05, and p>0.05, respectively) (Table 1). In addition, native thiol and total thiol of cyanotic patients were lower than in acyanotic patients (p<0.01 and p<0.05, respectively), while disulfide levels were significantly higher (p<0.01, Table 1). Disulfide/native thiol% and disulfide/total thiol% of cyanotic patients were higher than in acyanotic patients (p<0.05), while native thiol/total thiol% was significantly lower. The NLR of cyanotic patients was statistically higher than that of the acyanotic patients (p<0.01, Table 1).

Correlation analysis between parameters of preop cyanotic and acyanotic groups is listed in Table 2. OSI was shown positively correlated with TAS and TOS in the acyanotic condition (p<0.01 and p<0.05, respectively). NRL was positively correlated with disulfide, OSI, and ceruloplasmin in the cyanotic state (p<0.01, p<0.05, and p<0.01, respectively). NRL was negatively correlated with native thiol in the acyanotic state and total thiol in the cyanotic state (p<0.05). Native thiol in the acyanotic state was positively correlated with disulfide in the cyanotic state (p<0.01).

Table 2: Correlation analysis between oxidant status and thiol/disulfide homeostasis parameters of cyanotic and acyanotic groups.

Pairs	r	p-Value
OSI (acyanotic)-TAS (acyanotic)	−0.571	0.009[b]
OSI (acyanotic)-TOS (acyanotic)	0.531	0.016[a]
NRL (cyanotic)-OSI (cyanotic)	0.466	0.011[a]
NRL (cyanotic)-ceruloplasmin (cyanotic)	0.476	0.009[b]
NRL (acyanotic)-native thiol (acyanotic)	−0.485	0.030[a]
NRL (cyanotic)-total thiol (cyanotic)	−0.444	0.017[a]
NRL (cyanotic)-disulfide (cyanotic)	0.475	0.009[b]
Native thiol (acyanotic)-disulfide (cyanotic)	0.567	0.009[b]

NLR, neutrophil/lymphocyte ratio; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index. r and p are correlation coefficient and significance values. [a]Significant compared among groups p<0.05. [b]Significant compared among groups p<0.01.

Variables for surgery preop (baseline), at the postoperative 1st hour and 24th hour

Oxidant and antioxidant variables

As shown in Figure 1 and Table 3, The TAS levels of 1st hour were significantly higher than in the baseline and 24th hour (p<0.01, and p<0.05, respectively), while the TOS and OSI levels of the 24th hour were significantly lower than in the baseline and 1st hour (p<0.05, and p<0.001, respectively). MPO levels of the 24th hour were significantly lower than in the baseline and 1st hour (p<0.05, and p<0.01, respectively), while the ceruloplasmin levels of the 1st hour were significantly higher than in the baseline and 24th hour (p<0.05, and p<0.01, respectively). The albumin level in the baseline group was significantly higher than in the 1st hour (p<0.05). No statistically significant differences in IMA and prolidase were identified among the groups.

Table 1: Changes of oxidant status and thiol/disulfide homeostasis parameters in cyanotic and acyanotic state.

	Cyanotic (n=30)	Acyanotic (n=20)	p-Value
TAS, mmol Trolox eq/L	1.43 (1.31–1.59)	1.55 (1.37–1.77)	0.004[b]
TOS, μmol H_2O_2 eq/L	6.44 (4.09–8.73)	4.42 (3.97–5.77)	0.017[a]
OSI, arbitrary units	3.93 (2.83–5.67)	3.03 (2.05–3.79)	0.007[b]
MPO, U/L	137.8 (61.27–407.1)	84.98 (35.13–133.7)	0.010[a]
Ceruloplasmin, U/L	66.72 (43.94–118.4)	53.08 (35.14–80.52)	0.048[a]
Albumin, g/dL	3.88 (3.77–3.98)	3.97 (3.84–4.05)	0.013[a]
IMA, ABSU	0.87 (0.82–0.94)	0.83 (0.78–0.88)	0.014[a]
Prolidase, U/L	825.8 ± 140.6	873.7 ± 153.5	0.131[c]
NLR	1.75 (1.13–2.94)	1.06 (0.90–1.38)	0.007[b]
Native thiol, μmol/L	291 (255–366,3)	358 (302.6–377.2)	0.014[a]
Total thiol, μmol/L	324.4 ± 61.99	353.5 ± 40.6	0.036[a]
Disulfide, μmol/L	21.70 (17.95–30.50)	19.28 (15.45–22.33)	0.008[b]
Disulfide/Native thiol, %	7.69 (5.25–11.64)	6.91 (4.50–8.56)	0.019[a]
Disulfide/Total thiol, %	6.65 (4.98–9.47)	5.71 (4.18–6.47)	0.023[a]
Native Thiol/Total thiol, %	86.56 (80.59–90.96)	88.61 (87.81–92.84)	0.022[a]

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MPO, myeloperoxidase; IMA, ischemia modified albumin; NLR, neutrophil/lymphocyte ratio; IQR, interquartile range. Mann–Whitney U tests were performed because the other variables except for prolidase and total thiol variables were non-parametric variables. Normally distributed data were expressed as the mean ± standard deviation, and the data that were not normally distributed were expressed as the median (IQR; 25th–75th percentile). [a]Significant compared among groups p<0.05. [b]Significant compared among groups p<0.01. [c]Non-significant.

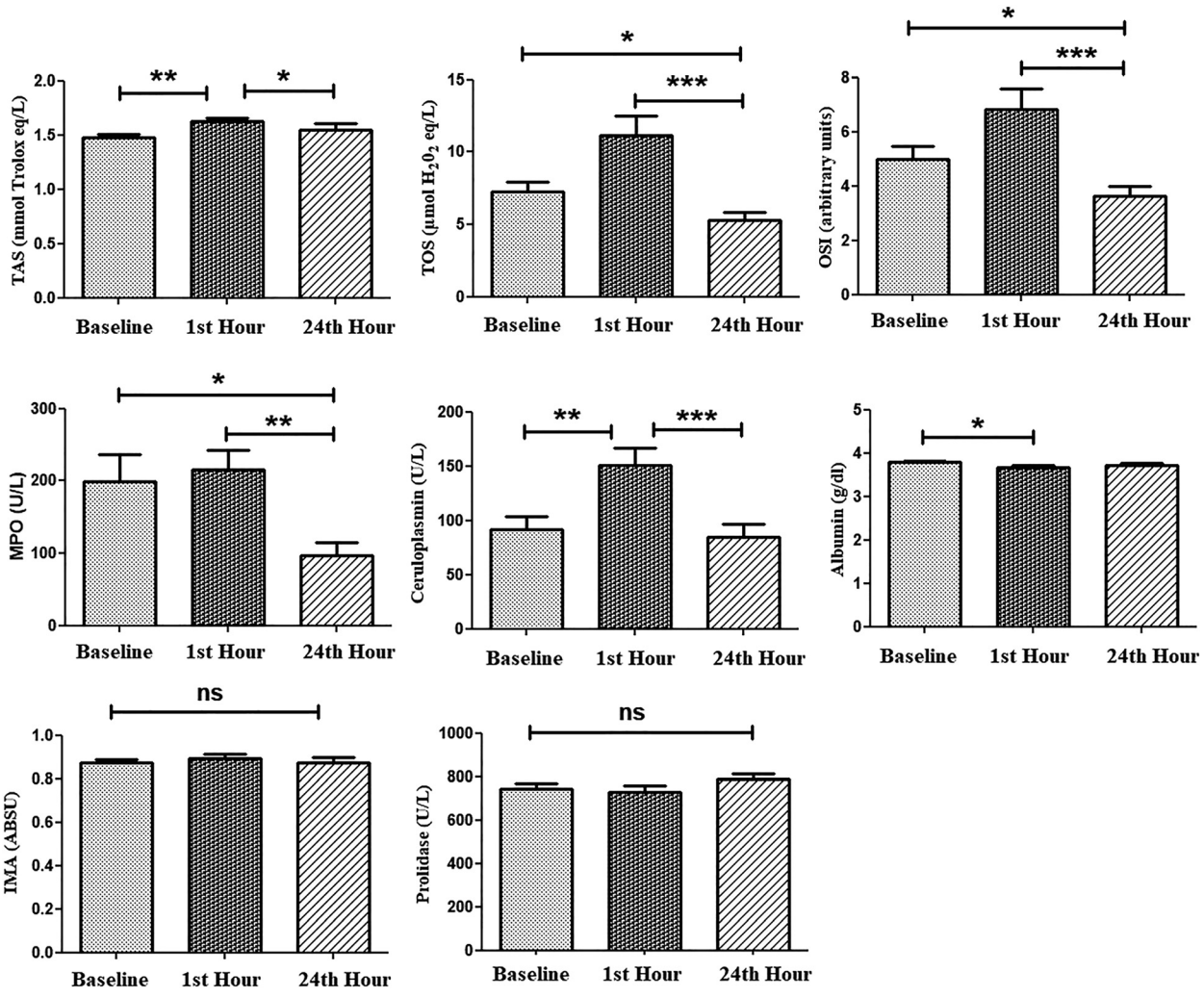

Figure 1: Oxidant and antioxidant variables. Baseline group (preop 1st hour); 1st hour (postop 1st hour); 24th hour (postop 24th hour). Since the results of all variables do not fit the normal distribution, the Kruskal–Wallis test with the post-hoc Dunn's multiple comparison test was applied. The data that were not normally distributed were expressed as the median (interquartile range (IQR); 25th–75th percentile). *p<0.05, **p<0.01, ***p<0.001; ns, non-significant.

Thiol-disulfide homeostasis variables

As shown in Figure 2 and Table 3, natural thiol levels in the baseline group were significantly lower than in the 1st hour and 24th hour (p<0.05 and p<0.01, respectively). Total thiol levels (μmol/L) in the baseline group were significantly lower than in 1st hour and the 24th hour (p<0.01 and p<0.001, respectively). Disulfide levels (μmol/L) in the 24th hour were significantly lower than in baseline (p<0.05). Disulfide/native thiol (%) and disulfide/total thiol (%) levels in the baseline group were significantly higher than in 1st hour (p<0.05 and p<0.01, respectively). Disulfide/native thiol (%) and disulfide/total thiol (%) levels in the baseline group were significantly higher than in the 24th hour (p<0.001 and p<0.05, respectively). No statistically significant differences in native thiol/total thiol (%) were identified among the groups.

Discussion

CHD is a crucial health problem worldwide, and it affects around nine out of every 1,000 live-born children. CHD is generally divided into two; these are acyanotic CHD (such as arterial, ventricular, atrioventricular septal defect) and cyanotic CHD (such as tetralogy of Fallot, transposition of the great arteries, truncus arteriosus) [13]. Our study investigated changes in thiol/disulfide balance and accompanying oxidative stress parameters according to pre-operative cyanotic and acyanotic status in pediatric

Table 3: Changes in thiol/disulfide balance and oxidative status parameters before and after surgery.

	Baseline (n=50)	1st hour (n=50)	24th hour (n=50)	p-Value
TAS, mmol Trolox eq/L	1.415 (1.318–1.605)	1.620 (1.495–1.770)	1.425 (1.250–1.778)	Group 1 vs. 2[b], Group 2 vs. 3[a]
TOS, μmol H_2O_2 eq/L	5.850 (3.908–8.590)	8.770 (4.615–12.68)	4.155 (3.495–5.073)	Group 1 vs. 3[a], Group 2 vs. 3[c]
OSI, arbitrary units	3.873 (2.920–6.313)	5.486 (2.916–8.149)	2.912 (2.308–3.703)	Group 1 vs. 3[a], Group 2 vs. 3[c]
MPO, U/L	120.8 (42.42–200.0)	188.4 (54.97–340.5)	53.14 (35.70–100.0)	Group 1 vs. 3[a], Group 2 vs. 3[b]
Ceruloplasmin, U/L	66.72 (34.70–124.4)	123.1 (77.14–160.2)	70.0 (33.53–106.2)	Group 1 vs. 2[b], Group 2 vs. 3[c]
Albumin, g/dL	3.895 (3.675–3.975)	3.765 (3.508–3.903)	3.780 (3.630–3.893)	Group 1 vs. 2[a]
IMA, ABSU	0.856 (0.808–0.918)	0.903 (0.843–0.952)	0.911 (0.835–0.958)	[d]
Prolidase, U/L	750.2 (656.2–890.4)	711.6 (572.9–823.8)	800.0 (691.2–902.1)	[d]
Native thiol, μmol/L	300.3 (295.5–367.5)	368.9 (303.3–402.3)	387.2 (295.2–414.5)	Group 1 vs. 2[a], Group 1 vs. 3[b]
Total thiol, μmol/L	339.3 (305.9–398.2)	406.5 (338.3–441.3)	423.6 (327.0–459.6)	Group 1 vs. 2[b], Group 1 vs. 3[c]
Disulfide, μmol/L	21.95 (17.79–30.58)	20.38 (15.23–26.10)	17.60 (12.53–26.33)	Group 1 vs. 3[a]
Disulfide/native thiol, %	7.680 (5.221–11.52)	5.534 (4.034–8.708)	4.945 (3.026–8.852)	Group 1 vs. 2[a], Group 1 vs. 3[c]
Disulfide/total thiol, %	6.543 (5.138–9.647)	4.898 (3.736–7.456)	5.003 (3.424–8.604)	Group 1 vs. 2[b], Group 1 vs. 3[a]
Native thiol/total thiol, %	87.09 (80.60–90.96)	90.66 (87.84–92.34)	90.30 (86.45–92.22)	[d]

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MPO, myeloperoxidase; IMA, modified albumin; IQR, interquartile range. Since the results of all variables do not fit the normal distribution, the Kruskal–Wallis test with the post-hoc Dunn's multiple comparison test was applied. The data that were not normally distributed were expressed as the median (IQR; 25th–75th percentile).
[a]Significant compared among groups p<0.05. [b]Significant compared among groups p<0.01. [c]Significant compared among groups p<0.001.
[d]Non-significant.

patients with CHD who underwent open-heart surgery for the first time. In this study, pre-operative TOS and OSI levels were higher in cyanotic CHD than in acyanotic CHD; on the contrary, the TOS level was lower. In addition, OSI levels in acyanotic patients correlate positively with TOS and negatively with TAS. There are a limited number of studies comparing the oxidative stress of children with CDH. A previous study found that lipid peroxidation and protein carbonyl levels were higher in patients with cyanotic CHD than acyanotic CDH and healthy controls. In addition to these, TAS levels decreased [14]. In another study, the oxidative status of cyanotic and acyanotic children with CHD was compared with healthy children, and TOS, TAS, and OSI levels were higher in cyanotic patients than in the acyanotic and control group [15]. As a first result, patients with CHD in the cyanotic state have increased oxidative stress due to hypoxia and anatomical defects. However, antioxidant response to increased oxidative stress has not been adequately provided.

In this study, pre-operative MPO, ceruloplasmin, IMA, and NLR levels were higher in cyanotic CHD than in acyanotic CHD; on the contrary, the albumin level was lower. In addition, NRL levels were positively correlated with both OSI and ceruloplasmin in cyanotic patients. In line with our findings, increased circulating MPO activities are generally associated with oxidative stress and inflammatory diseases (especially cardiovascular disease) [16]. Ceruloplasmin has ferroxidase function as well as copper transport, antioxidant and anti-inflammation activities. Ferroxidase catalyzes the formation of Fe+3 ions from Fe+2 ions, which are associated with antioxidant events. Ceruloplasmin ferroxidase activities were higher in valvular heart patients than in the healthy control group [17]. Hypoxia, inflammation, and free radical damage reduce the metal-binding capacity of albumin, resulting in the formation of IMA. IMA levels of patients with ventricular septal defects were higher than the healthy control group [18]. The decreased albumin level in our study may have also supported the increase in IMA level. Manuel et al. compared the pre-operative NLR levels of cyanotic and acyanotic CHD [19]. They found that cyanotic patients had a higher NLR, and these results were parallel with the NLR results of our study. As a second result, inflammation markers such as NLR, MPO, and ceruloplasmin increased due to decreased oxygen saturation and anatomical defects. In addition, decreased albumin levels, oxidative stress, and inflammation also support increasing IMA levels.

Prolidase, an iminodipeptidase, is responsible for regulating collagen metabolism (the extracellular matrix and vessel walls). Vascular damage results in collagen damage. It has been shown that prolidase activity is increased in coronary artery diseases [20]. In another study, the prolidase level was higher in obese patients than healthy controls [21]. As a different opinion, the TOS of patients with Bronchial Asthma increased, and prolidase activities decreased [22]. In our study, the prolidase level decreased under cyanotic conditions. We think that the

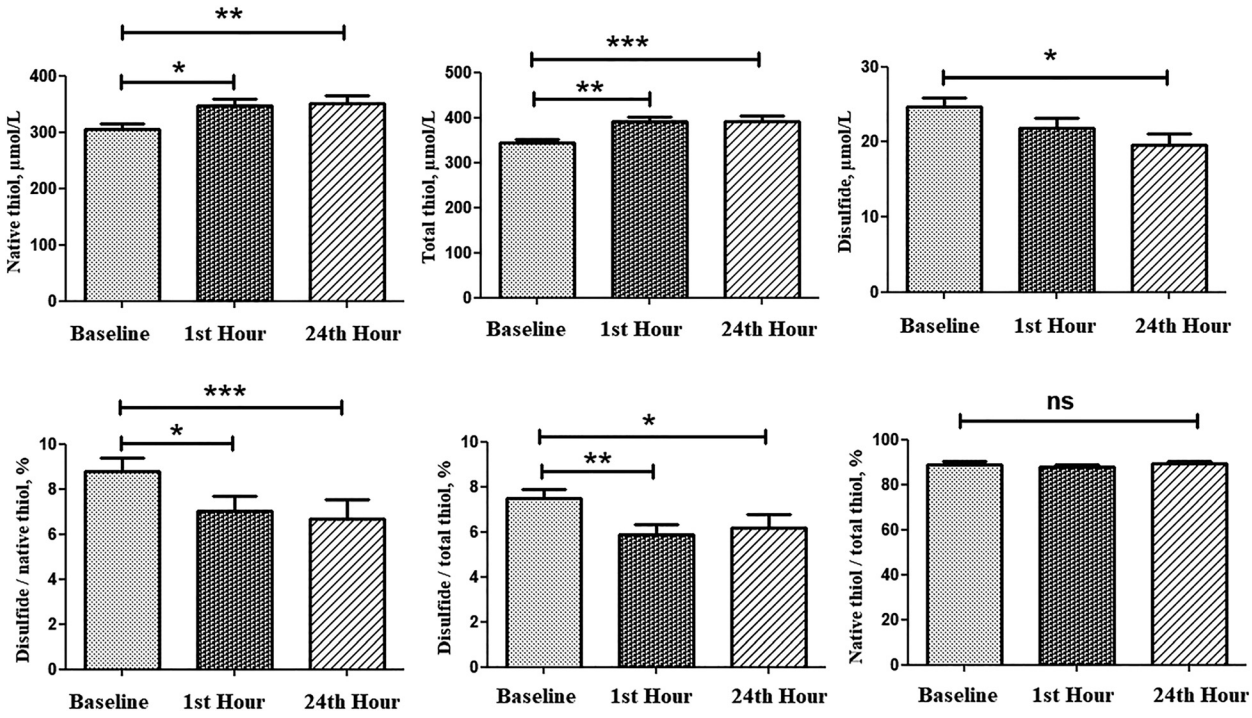

Figure 2: Thiol-disulfide homeostasis variables. Baseline group (preop 1st hour); 1st hour (postop 1st hour); 24th hour (postop 24th hour). Since the results of all variables do not fit the normal distribution, the Kruskal–Wallis test with the post-hoc Dunn's multiple comparison test was applied. The data that were not normally distributed were expressed as the median (interquartile range [IQR]; 25th–75th percentile). *p<0.05, **p<0.01, ***p<0.001; ns, non-significant.

reason for this is the decreased extracellular matrix production due to the malformation in cyanotic congenital heart patients.

In our results, native thiol and total thiol values of cyanotic patients were lower, and disulfide levels were higher than acyanotic patients. In addition, we found that NRL levels were negatively correlated with both native thiol and total thiol and positively correlated with disulfide. There is a study on thiol/disulfide homeostasis in CHD in which the natural thiol, total thiol, and disulfide levels were lower in children with ventricular septal defect than in children with tetralogy of Fallot [23]. Ventricular septal defect is often thought of as acyanotic CHD [13]. As a third result, the oxidative stress that increases due to CHD is tried to be neutralized with antioxidants in the body. Cysteine is the main factor forming the thiol/disulfide couple in human plasma. Thiols containing the sulfhydryl group (–SH) are sensitive to oxidative damage and reduce oxidative damage by becoming disulfide (–S–S). Our first and second results show that oxidative stress is increased in cyanotic CHD patients. Thiol groups, which are very sensitive to oxidant stress, are converted into reversible disulfide structures by the oxidant molecular. As the disulfide ratio formed by the combination of two thiol molecules increased, the native and total thiol ratios decreased, as seen in our results. The largest thiol-containing transporter in plasma is albumin, followed by glutathione, homocysteine, cysteine, and gamma globulin. In our study, the low level of albumin in cyanotics causes a decrease in thiol ratios.

The second part of our study, to our knowledge, is the first study to evaluate the changes in thiol/disulfide balance and oxidative stress parameters before and after surgery in pediatric patients with CHD who had undergone open-heart surgery. TAS, TOS, OSI, MPO, and ceruloplasmin levels were significantly higher at the post-operative 1st hour than the preoperative and post-operative 24th hour results. On the contrary, these parameters decreased at the post-operative 24th hour. In a previous study, pre- and post-operative TAS, albumin, bilirubin, uric acid, and high-sensitivity C-reactive protein levels were investigated in CHD patients. It was determined that the TAS levels of the patients who underwent cardiopulmonary bypass increased in the post-operative 1st hour and decreased in the 24th hour [4]. This result shows parallelism with the TAS results we found. In addition, 8-isoprostaglandin-F2α (8-iso-PGF2α) levels were measured as a marker of lipid peroxidation in children undergoing cardiac surgery, and 8-iso-PGF2α levels increased

in the 1st hour postoperatively and decreased in the 24th hour [24]. This result shows parallelism with the TOS, OSI, MPO, and ceruloplasmin results we found. During cardiac surgery, hemodilution between bypass circuits and blood cells causes inflammation. As a result, oxidative stress occurs [25]. It can also be thought that pediatric patients cannot respond effectively to oxidative stress because their antioxidant systems are not fully developed [26]. As a first result of the second part, it is seen that there is an increase in oxidative stress and inflammation in the 1st hours after the operation due to the operation and anatomical defects. In addition, during cardiac surgery, systemic inflammation, mechanical stress, intra-operative changes in blood pressure, perfusion, and hemodilution trigger oxidative stress.

Our findings suggest that the decrease in the albumin level in the post-operative 1st hour causes an increase in the IMA level due to the decrease in the metal binding capacity of albumin. In a previous study, IMA levels before and after coronary bypass operation were examined and intra-operative IMA level was higher than pre- and post-operative 24th hour IMA values [27]. In our study, the prolidase level increased at the post-operative 24th hour compared to the other groups. Prolidase levels of patients with previous coronary artery aneurysms were lower than the control group [28]. According to the preoperative situation, we can say that the increase in the post-operative prolidase level is caused by the disappearance of the anatomical defect and the increase in vascular and extracellular matrix formation.

Natural thiol and total thiol levels increased at the 1st and the 24th hours post-operatively. Conversely, disulfide levels decreased. On the other hand, there is no other published study examining pre- and post-operative thiol/disulfide homeostasis and oxidative stress parameters together in children with CHD. Previously, the preop thiol levels of children with CHD were examined, and it was found that the native- and total thiol levels of the control group were higher than the group with CHD [23]. In another study, the effects of thiol-disulfide homeostasis were examined in patients who underwent on-pump coronary artery bypass grafting, and it was found that native thiol, total thiol, and disulfide levels increased 6 h after the bypass [29]. As a second result of the second part, the possible mechanism of before the operation, CDH-induced cellular stress decreased both native and total thiol levels and increased disulfide levels. The imbalance of thiol-disulfide and increase in disulfide levels cause oxidative stress. Cellular stress that develops due to CDH after the operation has decreased, and thiol-disulfide has approached a state of equilibrium. In addition, primary solutions used during surgery (for example, fresh frozen plasma) and supplementation in the intensive care unit increased body temperature and blood production [30], which may have supported the increase in natural and total thiol levels. However, the low albumin level, one of our post-operative findings, does not support this claim.

The main limitation of this study is the single-center design, which represents our clinical experience with a small number of patients and the lack of longer follow-up of patients after the operation. Another limitation of this study is the use of anesthetic agents and blood products during the operation and the inability to interpret their effects on the study. Another important limitation of the study was the absence of a control group when differentiating according to oxygen saturation. Molecular, genetic, etiological, and more extensive studies are needed to support our findings of thiol-disulfide homeostasis in CHDs. It is recommended to expand the studies, especially considering it as a marker pre- and post-operative.

In conclusion, patients with CHD in the cyanotic state have increased oxidative stress (TOS and OSI levels) and inflammation (MPO, ceruloplasmin, IMA, and NLR levels) due to hypoxia and anatomical defects. Antioxidant response to increased oxidative stress has not been adequately provided. In the cyanotic state, thiol groups are converted into reversible disulfide structures by the molecular oxidant. As the disulfide ratio increased, the native and total thiol ratios decreased. Therefore, there is an increase in oxidative stress (TOS and OSI) and inflammation (MPO and ceruloplasmin) in the 1st hours after surgery due to surgical operation and anatomical defects. This increase decreased 24 h after the operation. In addition, cellular stress caused by CDH decreased both natural and total thiol levels before the operation; on the contrary, it increased disulfide levels. Thiol-disulfide imbalance and increase in disulfide level are markers of oxidative stress. After the operation, cellular stress due to CDH decreased and approached the thiol-disulfide equilibrium state. Finally, this is the first time that thiol-disulfide homeostasis is associated with markers of oxidative stress and inflammation in children with CHD.

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