The Oxidative Status of Children with Acyanotic Congenital Heart Diseases: A Randomized Controlled Study

Asiyanotik Doğumsal Kalp Hastalığı olan Çocuklarda Oksidatif Durum: Randomize Kontrollü bir Çalışma

Ayhan Pektaş[1], Bugra H. Koca[2], Bilgehan M. Pektaş[3], Resit Koken[4]

[1] Afyon Kocatepe University Hospital, Department of Pediatric Cardiology, Afyonkarahisar, Turkey
[2] Afyon Kocatepe University Medical Faculty, Department of Biochemistry, Afyonkarahisar, Turkey
[3] Afyon Kocatepe University Medical Faculty, Department of Pharmacology, Afyonkarahisar, Turkey
[4] Afyon Kocatepe University Hospital, Department of Pediatric Neurology, Afyonkarahisar, Turkey

ABSTRACT

Objective: This study aims to investigate the role of oxidative stress congenital heart defects with left-to-right shunt by determining total oxidant status (TOS), total antioxidant status and oxidative stress index (OSI) in affected children.
Methods: This is a prospective, randomized case-control study which reviews 40 healthy children and 40 children who have congenital heart defects with left-to-right shunt.
Results: The healthy children and the children who have congenital heart defects with left-to-right shunt are statistically similar with respect to age, sex, height, weight and body mass index (p>0.05 for all). Both groups have statistically similar echocardiography findings including interventricular septum thickness, left ventricular internal diameter, left ventricular posterior wall, systolic volume, end diastolic volume, fractional shortening, ejection fraction and left ventricular mass values (p>0.05 for each). These groups are also statistically similar in aspect of myocardial performance index, E´, A´, S´, relaxation time and contraction time values that have been specified for left ventricle, interventricular septum and right ventricle (p>0.05 for each). The healthy children and the children with congenital heart defects have statistically similar blood count parameters as well as serum concentrations of vitamin D and brain natriuretic peptide. The children who have congenital heart defects with left-to-right shunt have significantly higher TOS and OSI values than those of the healthy children (respectively, 35.6±2.8 nmol H_2O_2 equiv/mg protein vs 16.1±4.6 nmol H_2O_2 equiv/mg protein, p=0.018 and 32.4±1.4 vs 11.5±3.2, p=0.022).
Conclusion: The imbalance between the prooxidant and antioxidant reactions causes an enhancement in oxidative stress which may contribute to the pathogenesis of congenital heart defects with left-to-right shunting.

Key Words: Congenital heart defects, left-to-right shunt, oxidative stress

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ÖZET

Amaç: Bu çalışmada, soldan sağa şantlı doğumsal kalp hastalığı olan çocuklarda, total oksidan statü (TOS), total antioksidan statü (TAS) ve oksidatif stress indeksi (OSI) belirlenerek etkilenmiş çocukların oksidatif durumunun değerlendirilmesi amaçlanmaktadır.
Gereç ve Yöntem: Bu ileriye dönük, randomize, vaka-kontrol çalışmasında, 40 sağlıklı çocukla soldan sağa şantlı doğumsal kalp hastalığı bulunan 40 çocuk karşılaştırılmıştır.
Bulgular: Sağlıklı çocuklarla soldan sağa şantlı doğumsal kalp hastalığı bulunan çocuklar; istatistiksel olarak benzer yaşa, cinsiyete, vücut ağırlığına, boya ve vücut kitle indeksine sahiptir (hepsi için p>0.05). Bu iki grup, interventriküler septum kalınlığı, sol ventrikül iç çapı, sol ventrikül posterior duvar kalınlığı, sistolik hacim, diastol sonu hacim, fraksiyonel kısalma, ejeksiyon fraksiyonu ve sol ventrikül kitle değerleri gibi ekokardiyografi bulguları bakımından istatistiksel olarak benzerdir (her biri için p>0.05). Ayrıca, iki grup da, sol ventrikül, interventriküler septum ve sağ ventrikül için myokardial performans indeksi, E´, A´, S´, gevşeme zamanı ve kontraksiyon zamanı değerleri açısından istatistiksel olarak benzerdir (hepsi için p>0.05). sağlıklı çocuklarla soldan sağa şantlı doğumsal kalp hastalığı bulunan çocuklar; istatistiksel olarak benzer kan sayımı parametrelerine, serum D vitamini ve beyin natriüretik peptit konsantrasyonlarına sahiptir. Sağlıklı çocuklarla karşılaştırıldığında, soldan sağa şantlı doğumsal kalp hastalığı bulunan çocuklara ait TOS ve OSI değerleri anlamlı olarak yüksektir (sırasıyla, TOS için, 35.6±2.8 nmol H_2O_2 equiv/mg protein vs 16.1±4.6 nmol H_2O_2 equiv/mg protein, p=0.018 ve OSI için 32.4±1.4 vs 11.5±3.2, p=0.022).
Sonuç: Prooksidan ve antioksidan reaksiyonların arasındaki dengesizlik, oksidatif stres artışına yol açar ve bu artış, soldan sağa şantlı doğumsal kalp hastalıklarının patogenezine katkıda bulunabilir.

Anahtar Sözcükler: Doğumsal kalp hastalıkları, soldan sağ şant, oksidatif stres

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Yazışma Adresi / Address for Correspondence: Dr. Ayhan PEKTAŞ, Pediatrik Kardiyoloji Bilim Dalı, Afyon Kocatepe Üniversitesi Hastanesi, Afyonkarahisar, Türkiye Tel. +902722463333 Faks. +902722463322 E-posta: drayhanpektas@hotmail.com

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INTRODUCTION

Congenital heart defect can be defined as a problem in the structure of the heart which is present at birth. Congenital heart defects are usually evaluated in two groups: cyanotic and acyanotic heart defects. In the case of non-cyanotic heart defects, blood flows from the left side of the heart to the right side of the heart due to a structural deformity. Individuals with left-to-right shunting often retain normal levels of oxyhemoglobin saturation in systemic circulation. Common left-to-right shunt lesions include atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA), endocardial cushion defect (ECD) and partial anomalous pulmonary venous return (PAPVR). Left to right shunt causes an elevation of pulmonary blood flow, which triggers obstructive and obliterative alterations in the pulmonary vascular bed and a progressive increase in pulmonary vascular tone (1-4).

Oxidative stress occurs as a result of an imbalance between the oxidant status and the antioxidant defense mechanisms of a human body. It has been well established that oxidative stress induces apoptosis and the generation of reactive oxygen radicals which may be responsible for cellular damage (5-7).

Oxidative stress is a central mechanism of cellular damage that affects all organs and tissues. This imbalance between the oxidant status and anti-oxidant mechanisms is associated with several serious pediatric diseases such as bronchopulmonary dysplasia, respiratory distress syndrome, necrotizing enterocolitis, periventricular leukomalacia, hypoxic-ischemic encephalopathy and sudden infant death syndrome (8-10).

Since there are numerous oxidants and antioxidants in the body, measuring different oxidant and antioxidant molecules is impractical. That's why; measuring the total oxidant status (TOS) and total antioxidant status (TAS) is more valid and reliable (11). The present study aims to investigate the role of oxidative stress congenital heart defects with left-to-right shunt by determining TOS, TAS and oxidative stress index (OSI) in affected children.

MATERIALS and METHODS

The present study was approved by the Ethical Committee and Institutional Review Board of Afyon Kocatepe University Hospital, where it was conducted between January 2015 and January 2016. The parents of all participants gave written informed consent.

Patients

This is a cross-sectional, randomized case-control study which reviews 40 healthy children and 40 children who have congenital heart defects with left-to-right shunt. The healthy controls were randomly chosen from the children who were referred to the study center due to innocent murmurs during the study period. The pediatric patients with acyanotic congenital heart defects were also randomly chosen from the children who were diagnosed with left-to-right shunting during the study period. Randomization was performed by using sequentially numbered, sealed and opaque envelopes constructed from a random number table.

The children with any chronic disease, associated infections, elevated acute phase reactants, abnormal serum electrolytes, renal dysfunction and use of antioxidant medications were excluded. Data related with the age, gender, height and weight of the participants were recorded. Body mass index was calculated according to the following formula:

$$\text{Body mass index (kg/m}^2\text{)} = \text{Body weight (kg)} / \text{Body height}^2\ \text{(m}^2\text{)}$$

Echocardiography Examination

Echocardiography examination was performed by using equipment with 3- and 5-MHz transducers (Vivid S6, GE Healthcare, UK) between 24-48 hours after the diagnosis of ARF, and before the anti-inflammatory treatment was initiated. A standardized cross-sectional and Doppler echocardiography examination was performed with multiple orthogonal parasternal, apical and subcostal views with the patient in the left lateral decubitus position.

Laboratory Studies

Complete blood count including hemoglobin, mean corpuscular volume (MCV), red cell distribution width (RDW), leukocyte count, neutrophil/lymphocyte ratio, platelet count, and mean platelet volume (MPV) was made by automated counter (Coulter analyzer, Max Instruments Laboratory, Milan, Italy). Serum concentrations of vitamin D were measured by radioimmunoassay (DSL Diagnostic Systems Laboratories, USA), whereas serum levels of brain natriuretic peptide (BNP) were measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation (CVs) for vitamin D were 1.3% and 1.8% respectively, while the intra-assay and inter-assay CVs for BNP were 1.8% and 1.5% respectively.

The TOS of each supernatant fraction was determined using a novel automated measurement method that was also developed by Erel (12). The oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ions. The oxidation reaction is enhanced by glycerol molecules in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which is measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in nmol H_2O_2 equiv/mg protein.

The TAS of each supernatant fraction was determined using the novel automated measurement method developed by Erel (13). In this method, ferrous ion solution present in Reagent 1 is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, including the brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. Using this method, the antioxidant effect in the sample against these potent-free radical reactions that are initiated by the produced hydroxyl radicals is measured. The results are expressed as nmol Trolox equiv/mg protein.

The OSI value was computed by the following formula (14):

OSI (arbitrary unit) = TOS (mmol H2O2 Eqv./L) / TAC (mmol Trolox Eqv./L)

Statistical Analysis

Collected data were analyzed by Statistical Package for Social Sciences version 18.0 (SPSS IBM Software, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum-maximum) and categorical variables were denoted as numbers or percentages. Smirnov-Kolmogorov test was used to test the variable distributions. Student *t*-test, chi-square test and Mann Whitney U test were used for the comparisons. A post-hoc analysis was carried out to make a retrospective power analysis and a cohort size of 80 children (40 healthy children and 40 children who have congenital heart defects with left-to-right shunt) was found to have 64.6% power to detect a difference at the 0.05 significance level. Two-tailed p values less than 0.05 were accepted to be statistically significant.

RESULTS

The children who had congenital heart defects with left-to-right shunting consisted of 27 children with VSD, 8 children with ASD and 5 children with PDA. Table 1 compares the demographic characteristics of the healthy children and children who have congenital heart defects with left-to-right shunt. Both groups are statistically similar with respect to age, gender, height, weight and body mass index (p>0.05 for each). Fourteen children who had congenital heart defects with left-to-right shunt had Class I disease and the remaining 26 children had Class II disease, according to Ross classification. The mean systemic oxygen saturation of the children with left-to-right shunt was calculated to be 91.6±3.5 mmHg (range: 88-96 mmHg).

Table 1: Demographic Characteristics of the Study Cohort

	Congenital heart defects with left-to-right shunt (n=40)	**Healthy controls (n=40)**	**p**
Age (years)	1.32±0.09	1.36±0.40	0.188
Male/Female	20/20 (50%/50%)	18/22	0.190
Height (m)	0.66±0.01	(45%55%)	0.176
Weight (kg)	7.6±0.3	0.62±0.01	0.182
Body mass index (kg/m^2)	17.3±0.6	7.8±1.1 20.3±1.7	0.154

Table 2 demonstrates the echocardiography findings of the healthy children and the children with left-to-right shunting. Both groups have statistically similar fractional shortening, ejection fraction, systolic and end diastolic volumes, interventricular septum thickness, and left ventricle internal diameter, posterior wall thickness and mass values (p>0.05 for all).

Table 2: Echocardiography Findings of the Study Cohort

	Congenital heart defects with left-to-right shunt (n=40)	Healthy controls (n=40)	p
Interventricular septum end systole (cm)	0.89±0.07	0.99±0.08	0.192
Interventricular septum end diastole (cm)	0.66±0.02	0.72±0.03	0.164
Left ventricular internal diameter end systole (cm)	1.20±0.06	1.68±0.07	0.346
Left ventricular internal diameter end diastole (cm)	2.22±0.07	2.72±0.11	0.278
	0.89±0.03	1.11±0.05	0.355
Left ventricular posterior wall end systole (mm)	0.63±0.03	0.76±0.05	0.314
Left ventricular posterior wall end diastole (mm)	23.5±1.1	25.7±1.9	0.177
Systolic volume (ml)	36.3±1.3	33.4±2.7	0.136
End diastolic volume (ml)	45.5±1.2	39.9±1.4	0.288
Fractional shortening (%)	77.0±1.6	71.6±.8	0.169
Ejection fraction (%)	25.4±2.7	29.0±6	0.274
Left ventricular mass	59.8±7.6	63.6±5.2	0.326
Left ventricular mass index	0.75±0.05	0.72±0.02	0.205
Aorta/Left atrium	1.92±0.06	2.50±0.11	0. 222
Tricuspid annular plane systolic excursion	1.50±0.05	2.13±0.12	0.249
Mitral annular plane systolic excursion			

Table 3 presents the tissue Doppler echocardiography findings of the healthy children and the children who have congenital heart defects with left-to-right shunt. Both groups are statistically similar in aspect of myocardial performance index, E´, A´, S´, relaxation time and contraction time values that have been specified for left ventricle, interventricular septum and right ventricle (p>0.05 for all).

Table 3: Tissue Doppler Echocardiography Findings of the Study Population

	Congenital heart defects with left-to-right shunt (n=40)	Healthy controls (n=40)	p
Left ventricular myocardial performance index	56.9±2.3	54.1±2.2	0.148
	0.104±0.004	0.133±0.007	0.316
Left ventricle E´	0.067±0.004	0.071±0.004	0.119
Left ventricle A´	1.69±0.11	1.94±0.12	0.244
Left ventricle E´/A´	0.074±0.003	0.114±0.002	0.056
Left ventricle S´	46.2±1.9	50.3±1.8	0.124
Left ventricle relaxation time	49.7±1.7	54.4±1.9	0.119
Left ventricle contraction time	1.44±0.07	1.39±.13	0.216
Left ventricle ME/MA	58.4±2.5	50.0±2.2	0.220
Interventricular septum myocardial performance index	0.093±0.003	0.093±0.004	0.199
	0.069±0.004	0.064±0.003	0.165
Interventricular septum E´	0.061±0.003	0.055±0.002	0.174
Interventricular septum A´	46.2±1.9	45.4±2.2	0.188
Interventricular septum S´	53.1±1.5	49.5±1.7	0.159
Interventricular septum relaxation time	53.3±2.9	52.5±1.9	0.177
Interventricular septum contraction time	0.147±0.008	0.126±0.006	0.236
Right ventricle myocardial performance index	0.107±0.006	0.122±0.006	0.288
Right ventricle E´	1.54±0.13	1.29±0.1	0.328
Right ventricle A´	0.114±0.004	0.134±0.018	0.376
Right ventricle E´/A´	43.7±1.8	52.4±2.3	0.288
Right ventricle S´	52.1±1.7	53.8±2.0	0.269
Right ventricle relaxation time	14.8±1.5	11.8±1.4	0.414
Right ventricle contraction time			
Right ventricle TE/TA			

Table 4 displays the biochemical characteristics of the healthy children and the children who have congenital heart defects with left-to-right shunt. Both groups have statistically similar serum levels of vitamin D and BNP as well as blood count parameters including hemoglobin, MCV, RDW, leukocyte count, neutrophil/lymphocyte ratio, MPV, platelet count, platelet-to-lymphocyte ratio (p>0.05 for each).

Table 4: Biochemical Characteristics of the Study Cohort

	Congenital heart defects with left-to-right shunt (n=40)	Healthy controls (n=40)	p
Hemoglobin (g/dl)	11.6±0.2	11.6±0.3	0.199
Mean corpuscular volume (fl)	77.7±1.2	78.1±1.7	0.183
Red cell distribution width (%)	16.2±0.9	14.7±0.3	0.144
Leukocyte count ($x10^3/mm^3$)	11.4±0.5	12.3±0.5	0.125
Neutrophil/Lymphocyte count	0.68±0.01	0.42±0.03	0.216
Platelet count ($x10^3/mm^3$)	413.7±19.6	456.2±17.1	0.266
Platelet/Lymphocyte count	53.4±11.2	57.7±16.5	0.284
Mean platelet volume (fl)	7.3±1.4	7.7±0.1	0.194
Vitamin D (ng/ml)	29.6±3.1	29.4±2.6	0.113
Brain natriuretic peptide (pg/ml)	57.9±3.6	65.6±3.4	0.236
Total oxidant status (nmol	35.6±2.8	16.1±4.6	0.018*
H_2O_2 equiv/mg protein)	1.1±0.2	1.4±0.3	0.137
Total antioxidant status (nmol	32.4±1.4	11.5±3.2	0.022*
Trolox equiv/mg protein)			
Oxidative stress index			

*p<0.05 was accepted to be statistically significant.

The children who have congenital heart defects with left-to-right shunt have significantly higher TOS and OSI values than those of the healthy children (respectively, 35.6±2.8 nmol H_2O_2 equiv/mg protein vs 16.1±4.6 nmol H_2O_2 equiv/mg protein, p=0.018 and 32.4±1.4 vs 11.5±3.2, p=0.022). However, both the healthy children and the children with congenital heart defects had statistically similar TAS values.

DISCUSSION

The pathogenesis of congenital heart defects is complex and involves genetic, inflammatory and autoimmune mechanisms. In patients with uncorrected left-to-right shunts, increased pulmonary pressure leads to vascular remodeling and endothelial dysfunction, secondary to an imbalance in vasoactive mediators which promotes vasoconstriction, inflammation, thrombosis, cell proliferation, impaired apoptosis and fibrosis (15). Aburawi et al. determined that inflammation decreases coronary flow in children with acyanotic heart defects (16).

The altered hemodynamic state of the children who have congenital heart defects with left-to-right shunt has been investigated by developing a lamb model with an in utero placement of an aorto-pulmonary vascular graft. Therefore, early disruption in reduction-oxidation balance and appearance of oxidative stress within pulmonary vasculature has been considered to be essential for the occurrence of endothelial dysfunction and vascular remodeling (17).

Previously published studies have focused on the use for the management of pulmonary hypertension, which is a late term complication of congenital heart defects with left-to-right shunts. The results of these clinical trials are not very promising, but a thorough identification of the temporal regulation of reactive oxygen species participating in the pathogenesis of congenital heart defects may help to achieve better clinical outcomes (18).

Karatas et al. assessed 25 children with rheumatic heart valve diseases, 25 children with congenital heart valve diseases and 20 healthy age-matched control subjects. The levels of plasma TAS, TOS and OSI values were found to be statistically similar in all groups. In addition, there were no correlations between the TOS and OSI values in the study cohort. Hence, it was declared that oxidative stress had no pronounced effect on the etiopathogenesis of congenital heart valve disorders during childhood (19).

Rokicki et al. evaluated the oxidant and antioxidant status in 23 infants suffering from congenital heart diseases (14 with left-to-right shunt and 9 with cyanotic heart defects) and 18 healthy infants. The study was based on the measurement of the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in blood, the levels of low molecular weight antioxidants (vitamin E, uric acid and selenium) and the concentration of malondialdehyde as a marker of lipid peroxidation. These three groups had statistically similar activities of superoxide dismutase and catalase. When compared to the healthy controls, infants with acyanotic congenital heart diseases had significantly lower plasma vitamin E levels. The activity of glutathione peroxidase was significantly lower and the levels of oxidant molecules (malondialdehyde and uric acid) were significantly higher in infants with cyanotic heart defects than in the healthy controls (20).

Ercan et al. conducted a case-controlled, cross-sectional study on 32 healthy children, 30 children with acyanotic heart disease and 29 children with cyanotic heart disease. They reported that the plasma TAS, TOS and OSI were significantly higher in children with cyanotic heart diseases than in children with acyanotic heart diseases and healthy controls. However, they were unable to detect significant differences between the TAS, TOS and OSI values of the healthy controls and children with acyanotic heart diseases (21).

Vitamin D can be considered as a casual antioxidant which scavenges reactive oxygen species in the first stage before activation of other stress-sensitive response pathways. It has been also shown that vitamin D levels are directly associated with anti-oxidant glutathione reductase and inversely associated with oxidant status markers such as superoxide dismutase and glutathione peroxidase in both healthy and diabetic adults (22, 23). However, this study failed to find a significant difference in vitamin D concentrations of the healthy children and the children who have congenital heart defects with left-to-right shunt.

To the best of our knowledge, this is the first randomized controlled study which aims to define the oxidant and antioxidant status of the children who have congenital heart diseases with left-to-right shunting. The present study compares 40 children with acyanotic heart defects and 40 healthy children who are matched with respect to age, gender and body mass index. Although a significant increase was specified in the TOS and OSI values of the children with acyanotic congenital heart diseases, no significant alteration can be indicated in their plasma TAS levels and serum vitamin D concentrations. This failure can be explained by the violation of the equilibrium between the oxidant and anti-oxidant mechanisms.

It has been demonstrated that oxidative stress plays a significant role in the pathogenesis of inflammatory disorders. An increase in oxidative stress induces apoptotic cell death and exerts negative effects on the immune system functions (24). The mechanism by which oxidative stress induces inflammation and vice versa is unclear but is of great importance, being apparently linked to many chronic inflammatory diseases (25). It has been also shown that ozone produces an initial direct injury to cell membranes, which may result in increased oxidative stress, which possibly affects macrophages and results in increased release of chemotactic stimuli for neutrophils. The consequent secondary inflammatory response may result in further cell injury and increased permeability (26). A complete blood count consists of inflammatory markers which include MCV, RDW, and MPV, leukocyte count, neutrophil/lymphocyte ratio and platelet/lymphocyte ratio (27, 28). That's why; this study has evaluated the healthy children and the children with congenital left-to-right shunting in aspect of these inflammatory markers and no significant difference could be detected. Such discrepancy may be attributed to the relatively small cohort size and mild-to-moderate progression of the congenital defect-related heart failure.

The findings of this randomized controlled study imply that the imbalance between the prooxidant and antioxidant reactions causes an enhancement in oxidative stress which contributes to the pathogenesis of congenital heart defects with left-to-right shunting. However, these findings should be interpreted carefully as there are several factors that limit their power. These factors include the relatively small cohort size, absence of children with cyanotic congenital heart disease and lack of longitudinal data.

Further research is warranted to clarify the role of oxidative stress in congenital heart defects with left-to-right shunts that affect children.

Conflict of interest

No conflict of interest was declared by the authors

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