The oxidative state of children with cyanotic and acyanotic congenital heart disease

Siyanotik ve asiyanotik doğumsal kalp hastalığı olan çocuklarda oksidatif durumun değerlendirilmesi

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Abstract

Objective: The type of congenital heart disease, early diagnosis, and treatment modality all play an important role in the morbidity and mortality of these diseases. This study examined the relationship between congenital heart disease and oxidative stress in children with cyanotic and acyanotic congenital heart disease.

Methods: In this case-controlled cross-sectional study, the study groups consisted of 29 patients with cyanotic heart disease, 30 patients with acyanotic heart disease, and a control group of 32 healthy individuals. For all groups, the total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) were calculated. Of the cyanotic group, 12 were diagnosed with tetralogy of Fallot, 7 with transposition of the great arteries, 4 with tricuspid atresia, 4 with double outlet right ventricle and 2 with truncus arteriosus. In the acyanotic group 19 patients had ventricular septal defect (VSD), 5 atrial septal defect (ASD) and 6 patent ductus arteriosus (PDA). Statistical analyses were performed using Chi-square, Mann-Whitney U and Kruskal-Wallis tests.

Results: The plasma TAS, TOS, and OSI were significantly higher in the cyanotic group than in the acyanotic group ($p<0.0001$, $p<0.01$, and $p<0.01$, respectively) and controls ($p<0.0001$, $p<0.0001$, and $p<0.0001$, respectively). A comparison between the acyanotic and control groups showed no statistical differences.

Conclusion: The level of oxidative stress in patients with cyanotic congenital heart disease was significantly higher than in the acyanotic and control groups, which were similar. (*Anadolu Kardiyol Derg 2009; 9: 486-90)*

Key words: Child, congenital heart diseases, cyanotic, acyanotic, oxidative stress

Özet

Amaç: Doğumsal kalp hastalıklarının cinsi, erken tanısı ve uygulanan tedavi yöntemi hastalığın morbidite ve mortalitesinde önemli rol oynamaktadır. Bu çalışmada siyanotik ve asiyanotik doğumsal kalp hastalığı olan çocuklarda, doğumsal kalp hastalığı ile oksidatif stres arasındaki ilişki araştırıldı.

Yöntemler: Bu vaka-kontrollü, enine-kesitsel araştırmada çalışma grubu 29 siyanotik, 30 asiyanotik doğumsal kalp hastalıklı hasta ve sağlıklı 32 kişinin olduğu kontrol grubundan oluştu. Tüm gruplarda total oksidan seviye (TOS) ve total antioksidan seviye (TAS) çalışıldı ve oksidatif stres indeks (OSİ) değerleri hesaplandı. Siyanotik gruptaki hastaların 12'si Fallot tetralojisi, 7'si büyük arter transpozisyonu, 4'ü triküspit atrezisi, 4'ü çift çıkımlı sağ ventrikül ve 2'si trunkus arteriosus tanısı aldı. Asiyanotik gruptaki hastaların ise en büyük kısmını VSD oluşturuyordu; bu hastalardan 19 tanesi VSD, 5'i ASD ve 6 tanesi de PDA tanısı aldı. İstatistiksel analizde Ki-kare, Mann-Whitney U ve Kruskal Wallis testleri kullanıldı.

Bulgular: Siyanotik doğumsal kalp hastalıklı grup ile kontrol grubunda bakılan plazma TOS, TAS, OSI düzeyleri karşılaştırıldığında siyanotik grupta anlamlı derecede yüksek bulundu (sırayla $p<0.0001$, $p<0.0001$, $p<0.0001$). Siyanotik doğumsal kalp hastalıklı grup ile asiyanotik grup plazma TOS, TAS, OSI düzeyleri değerlendirildiğinde siyanotik grupta anlamlı derecede yüksek bulundu (sırayla $p<0.0001$, $p<0.01$, $p<0.01$). Asiyanotik ve kontrol grubu arasında TOS, TAS, OSI değerlerinde istatistiksel olarak fark yoktu.

Sonuç: Oksidatif stres, siyanotik doğumsal kalp hastalığı olan hastalarda asiyanotik ve kontrol grubu hastalarına göre belirgin olarak yüksek, asiyanotik doğumsal kalp hastalığı olan hastalarda ise sağlıklı çocuklara yakın düzeyde bulundu. *(Anadolu Kardiyol Derg 2009; 9: 486-90)*

Anahtar kelimeler: Çocuk, doğumsal kalp hastalığı, siyanotik, asiyanotik, oksidatif stres

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Introduction

The limited number of published studies comparing the oxidative status of children with cyanotic and acyanotic congenital heart disease with that of healthy children shows the necessity for a study of the oxidative status of these patients.

The effects of environmental and genetic factors result in the multifactorial etiology of congenital heart disease, which is seen in 0.8~1% of Turkish school children (1). The dark coloration associated with deoxygenated hemoglobin in the blood causes cyanosis, and right-to-left shunting is an important clinical finding in congenital heart disease (2).

The body produces energy by burning carbohydrates and fat; in other words, oxidation must take place. These metabolic reactions normally result in the formation of free oxygen radicals, which play a role in the defense against foreign substances and infectious agents (3, 4). The body maintains a balance between the various pre-oxidative factors that affect the system and the antioxidant system created to counteract these. When excessive oxidative stress occurs, the body responds to restore harmony balance. With ischemia, reperfusion, and chronic hypoxia, patients with congenital heart disease unlike normal individuals, cannot adequately meet the biological needs of tissues and are exposed to excess oxygen radicals, which cause deterioration in the patient's condition leading to difficulties in treatment (5). Rokicki et al. (6) examined the relationship between congenital heart disease and oxidative stress; there are a few other studies on the subject (7, 8). The study group examined by Rokicki et al. (6) consisted of children with cyanotic and acyanotic congenital heart disease but it was a very small group. In that study, when antioxidant activity was compared with the healthy controls, no significant difference was found. In another study (7), together with many other parameters, the oxidative stress markers were examined in pregnancy of babies found to have congenital heart defects and the mothers who did not. To the best of our knowledge in literature there is one study on children and that study was carried out on a very small patient group. Moreover, as there is no study showing how the total oxidative status is affected in children with cyanotic and acyanotic congenital heart disease, this has become an important topic.

This study examined the relationship between congenital heart disease and oxidative stress by measuring the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of children with cyanotic congenital heart disease.

Methods

Patients in this case-controlled cross-sectional study had been diagnosed with congenital heart disease after presenting at the Harran University Medical Faculty, Department of Pediatric Cardiology. Congenital heart disease was diagnosed by echocardiography or angiography. Two study groups were formed, one consisting of 29 cyanotic and the other of 30 acyanotic patients. The control group consisted of 32 healthy patients whose echocardiography results were normal.

Patients were excluded from the study if findings showed septicemia, pulmonary disease, hypoxia/anoxia, other congenital anomalies, chromosomal anomalies, metabolic disease, cephalohematoma, ecchymosis, polycythemia, and systemic disease or any other disease that would cause free radicals to form.

The study protocol was approved by the local ethics committee. The details of the study were explained, and all the participants provided informed consent.

Measurements

The subjects were told not to eat, drink, or take any antioxidant medicine for 3 hours before the blood samples were collected.

Samples

Blood samples were withdrawn into heparinized tubes from a cubital vein and then stored immediately in ice. Plasma was separated from cells by centrifugation at 3000 rpm for 10 min. The plasma samples were stored at -80°C until analysis.

Measuring the total antioxidative capacity of plasma (TAS)

The total antioxidant status (TAS) of the plasma was determined using a novel automated measurement method developed by Erel (9). In this method, hydroxyl radical, which is the most potent biological radical, is produced. The assay measures the antioxidative effect of the sample against potent free radical reactions that are initiated by the hydroxyl radical produced. The precision of the assay is excellent, and is lower than 3%. The results are expressed as μmol Trolox equivalent/L.

Measuring total oxidant status (TOS)

The total oxidant status (TOS) of serum was determined using a novel automated measurement method, as described previously (10). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by the glycerol molecules present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in acidic solution. The color intensity, which was measured spectrophotometrically, is proportional to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 equivalent/L).

Oxidative stress index (OSI)

The percentage ratio of the total peroxide level to the TAC was used as the oxidative stress index (OSI) (11). The OSI was calculated as the total peroxide (in μmol/L) divided by the TAC (μmol Trolox equivalent/L) divided by 100.

None of the subjects was taking any drug known to affect lipid or lipoprotein metabolism. Special care was taken to exclude subjects who were taking anabolic drugs, vitamins, or other antioxidants, or who were smokers. None of the subjects was following a special diet.

Statistical analysis

The data were analyzed using SPSS® for Windows version 11.5 (Chicago, IL, USA). The values are expressed as the mean ± SD for the congenital heart disease (cyanotic and acyanotic) groups and the healthy population separately. The Kolmogorov-Smirnov test was used to verify whether the data were distributed normally. Qualitative variables were assessed using the Chi-square test. The differences between groups were analyzed using the Kruskal-Wallis test and Mann-Whitney *U* test. $p<0.05$ was accepted as significant.

Results

The demographic details of the patient and control groups are given in Table 1. No statistically significant differences were found between the groups in terms of age or gender distribution ($p>0.05$).

The diagnoses of congenital heart disease for both the cyanotic and acyanotic groups are shown in Table 2. The age range of the cyanotic group was 1-11 months with a mean age at diagnosis of 3.7±2.8 months, and the mean age at diagnosis of the acyanotic group was 7.5±21.4 months. The difference in the age at diagnosis between the cyanotic and acyanotic groups was not significant.

The results of comparison of oxidative and antioxidative parameters are presented in Table 3. The TOS value of the cyanotic group compared to the acyanotic group and the control group was higher ($p<0.0001$). The TAS value of the cyanotic group compared to the acyanotic group and the control group was higher ($p<0.0001$). The OSI value of the cyanotic group compared to the acyanotic group and the control group was higher ($p<0.0001$). The TOS, TAS, and OSI of the cyanotic, acyanotic, and control groups are shown in Figures 1-3.

Discussion

In this study the oxidant and antioxidant values of the cyanotic congenital heart disease group were higher than were those of the acyanotic and control groups, whereas no significant differences were found between the acyanotic and control groups.

The dark purple coloration of the lips, nail base, tongue, and oral mucosa (central cyanosis) of patients with cyanotic congenital heart disease is the main reason that they seek medical help in the early neonatal period. These babies are diagnosed at an earlier age than those whose congenital heart disease is not cyanotic. In our study, although no statistically significant difference was found between the cyanotic and acyanotic groups in terms of the age at diagnosis, the patients in the cyanotic group were diagnosed earlier (Table 1). Free radicals are reactive compounds that are produced naturally in the body. They can have positive (e.g., in the immune system) or negative (e.g., lipid, protein, or DNA oxidation) effects on the body. To limit the harmful effects, the organism needs a strong antioxidant system. This system consists of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase,

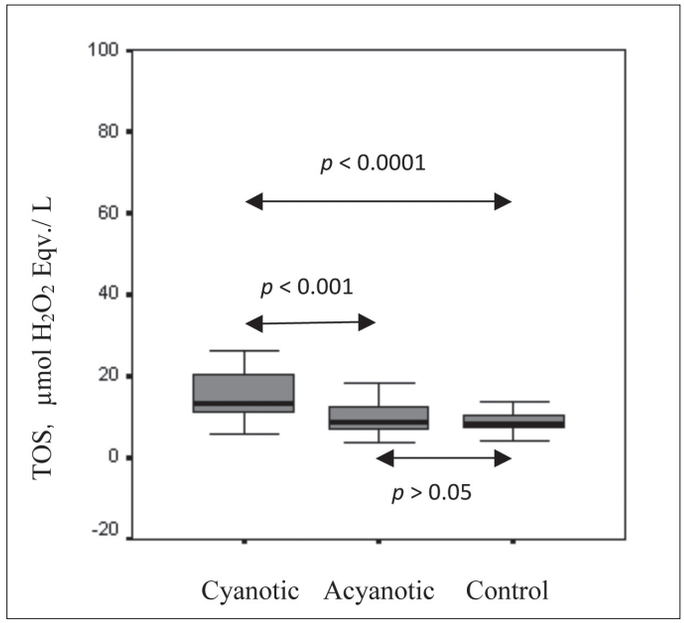

Figure 1. TOS values (mean ± SD) for the cyanotic, acyanotic, and control groups

TOS - total oxidant status, Mann - Whitney U test

Table 2. Diagnosis of congenital heart disease for both the cyanotic and acyanotic groups

Cyanotic group	Number	Acyanotic group	Number
Tetralogy of Fallot	12	Ventricular septal defect	19
Transposition of the great arteries	7	Atrial septal defect	5
Tricuspid atresia	4	Patent ductus arteriosus	6
Double outlet right ventricle	2		
Truncus arteriosus	2		
Total	29		30

Table 1. Demographic and clinical characteristics of the cyanotic, acyanotic patients and control groups

Variables	Cyanotic group (n=29)	Acyanotic group (n=30)	Control group (n=32)	Chi- square	p
Sex, male/female*	17/12	12/18	16/16	0.35	>0.05
Age, months* *	30.2±46.1	29.4±42.3	32.3±40.4	0.87	>0.05
Age at diagnosis, months**	3.7±2.8	7.5±21.4	-	0.74	>0.05

Values are expressed as the mean ± SD

*Chi-square test , **Kruskal–Wallis test

Table 3. Pairwise comparisons of the oxidative and antioxidative parameters in cyanotic, acyanotic patients and the control groups

Variables	Cyanotic group (n=29)	Acyanotic group (n=30)	Control group (n=32)	* Chi-square	p
TOS, µmol Trolox equivalents /L	** 20.04±16.70	9.93±3.70	9.2±3.5	20.02	<0.0001
TAS, µmol H_2O_2 equivalents/ L	** 2.32±0.14	2.17±0.25	2.10±0.20	19.20	<0.0001
OSI, unit	** 8.62±7.32	4.63±1.73	4.35±1.47	15.71	<0.0001

Values are expressed as the mean ± SD
* Kruskal-Wallis test
OSI - oxidative stress index, TAS - total antioxidant status, TOS - total oxidant status

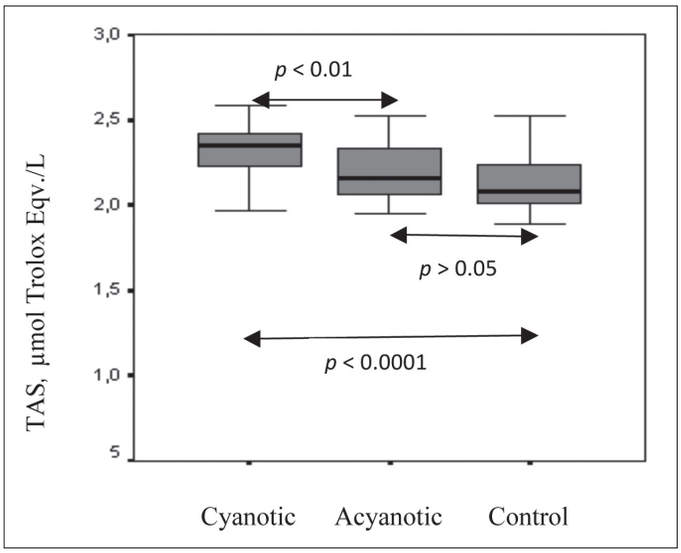

Figure 2. TAS values (mean ± SD) for the cyanotic, acyanotic, and control groups
TAS - total antioxidant status, Mann - Whitney U test

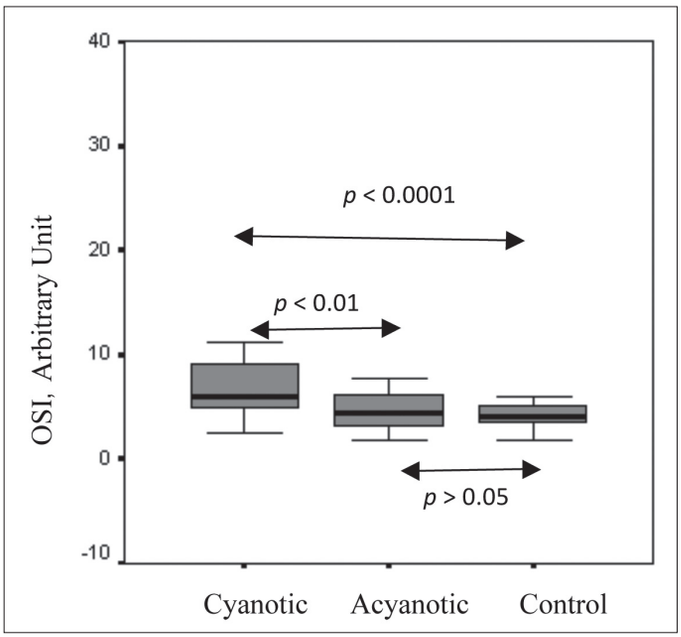

Figure 3. OSI values (mean ± SD) for the cyanotic, acyanotic, and control groups
OSI - oxidative stress index, Mann - Whitney U test

vitamin A (retinoic acid), vitamin E (alpha-tocopherol), vitamin C (ascorbic acid), uric acid, and glutathione. An imbalance between the oxidant and antioxidant systems can lead to a state of oxidative stress and various diseases in later life (e.g., Parkinson's disease and cancer) (12). Under normal circumstances, a functional antioxidant system protects cells from damage by free oxygen radicals. This keeps the oxidant and antioxidant systems of the organism in balance. When a decrease occurs in one antioxidant, an increase in another can compensate. If the benefits of pro-oxidants and oxidants are lost, leukocytes produce inflammatory mediators and free oxygen radicals. In this way, lipid peroxidation occurs in the cell membranes causing cell damage and disease. The cell membranes are very sensitive to free oxygen radicals, especially those of erythrocytes (13, 14). Studies of the effects of oxidative stress in pregnancy have suggested a relationship between congenital heart disease and oxidative stress (teratogenic effect) (7, 8). Importantly, these studies showed that oxidative stress in pregnancy contributes to congenital heart disease. As few studies are available supporting this, further research is needed. In a study of 41 infants and newborns with cyanotic or acyanotic congenital heart disease, decreased oxidant and antioxidant substances were much more evident in those with cyanotic congenital heart disease (6). These findings concur with our results. To our best knowledge, no other published study has studied oxidative stress in infants with congenital heart disease. In our study, rather than categorizing the patients according to age, the patients with congenital heart disease were subdivided into a group with cyanotic and a group with acyanotic congenital heart disease, and the oxidative stress status was compared with a control group. The results showed that the TOS, TAS, and OSI of the cyanotic congenital heart disease group were higher than were those of the acyanotic and control groups, whereas no significant differences were found between the acyanotic and control groups (p>0.05).

Free oxygen radicals play an important role in tissue damage with inadequate blood circulation (6). According to our results, the hypoxia that develops is dependent on the underlying anatomical defects of these patients and increases the secondary free oxygen radicals; it is possible that the levels of antioxidant substances in the body are increased to compensate for this. The increased free oxygen radicals, which depend on the degree of chronic hypoxia in cyanotic congenital heart disease, lay the foundations for several diseases such as

atherosclerosis (15, 16). This indicates the importance of applying palliative or corrective techniques at the earliest age possible for patients with cyanotic congenital heart disease.

Acyanotic congenital heart disease patients are under less oxidative stress than are those with cyanotic congenital heart disease. Nevertheless, care must be taken in the treatment of these patients to deal with oxidative stress problems that may arise. Appropriate treatment must be applied promptly for patients with acyanotic congenital heart disease according to their clinical condition. Some patients may not require any intervention, and except for those with a secundum type atrial septal defect, these patients are simply observed and given infective endocarditis prophylaxis (2). In such patients, the body compensates for the existing pathology, and perhaps after a certain period, the pathological condition that has brought about an increase in oxidative stress may be suppressed or brought under control by antioxidants, which have increased in response to that condition. Therefore, the oxidative stress index of such patients may remain normal.

Study limitations

One of the limitations of our study was the relatively small sample size of the groups. Another important limitation of this study is that the oxidative status of the cyanotic congenital heart disease patient group was not evaluated before and after surgery or intervention.

Conclusion

In conclusion, we found that the level of oxidative stress in patients with cyanotic congenital heart disease was significantly higher than in those with acyanotic congenital heart disease and healthy controls, whereas the levels in the last two groups were similar.

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