Endothelial dysfunction and atherosclerosis in children with irreversible pulmonary hypertension due to congenital heart disease

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ABSTRACT

Objective: : **To assess endothelial dysfunction and the risk for coronary atherosclerosis in children with irreversible pulmonary hypertension due to congenital heart disease (CHD).**

Methods: : **The study included 18 cyanotic patients (the mean age was 12.28 ± 3.26 years) who developed irreversible pulmonary hypertension due to cyanotic and acyanotic CHDs, and 18 control patients (the mean age was 11.78 ± 3.00 years). Study groups were compared for flow-mediated dilatation (FMD), carotid intima media thickness (CIMT) and atherosclerotic risk factors.**

Results: : **Compared to the control group, the mean FMD was significantly reduced in the cyanotic group (5.26 ± 2.42% and 9.48 ± 2.60%, respectively; *P*-value < 0.001). No significant difference was observed between the groups in CIMT (0.41 ± 0.08 mm and 0.39 ± 0.06 mm, respectively; *P*-value = 0.299). The levels of total cholesterol, low-density lipoprotein–cholesterol and very low-density lipoprotein–cholesterol were statistically significantly lower compared tothe control group (*P*-value = 0.001, 0.006 and 0.014, respectively), whereas no statistically significant difference was found in the levels of high-density lipoprotein–cholesterol and triglycerides (*P*-value = 0.113 and 0.975, respectively).**

Conclusions: : **Systemic endothelial dysfunction in children with irreversible pulmonary hypertension due to CHD was noted but there was no increased risk for atherosclerosis,.**

Keywords: : Childhood, congenital heart disease, flow-mediated dilatation, pulmonary hypertension

INTRODUCTION

Eisenmenger syndrome (ES) is described as reversal of the initial left-to-right cardiac shunt because of the elevated pulmonary artery pressure (PAP) and pulmonary vascular resistance at or above the systemic level.[1] ES presents with chronic hypoxemia due to venous to arterial mixing at the atrial, ventricular or arterial level.[2] Chronic hypoxemia results in significant alterations in the structure and function of the vessels. Increased erythrocytosis and blood viscosity secondary to the chronic hypoxemia cause changes in nitric oxide metabolism, leading to endothelial dysfunction in cyanotic congenital heart diseases (CHD). Endothelial vascular tone plays a role in angiogenesis and homeostasis, and it represents the most damaged vascular layer in the presence of hypoxia. Endothelium-derived relaxation factor is released from the endothelial cells, regulating relaxation of the vascular smooth muscle cells. These regulatory mechanisms are disturbed in case of endothelial dysfunction.[3]

As markers of the endothelial dysfunction in CHD in relation to the ES, the von Willebrand factor antigen and tissue-type plasminogen activator are increased, and thrombomoduline level is reduced.[4] In CHD with pulmonary hypertension, plasma endothelin-1 (ET-1) concentrations are increased.[5] ET-1 has a major role in the regulation of basal vascular tone.[6,7] And, it is likely that ET-1 is involved in impairment of the nitric oxide-dependent vasodilatation.[7,8]

Systemic endothelial dysfunction is a prognostic marker in acute coronary syndrome and coronary atherosclerosis.[9,10] Besides systemic endothelial dysfunction, isolated

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coronary vascular endothelial dysfunction is also an independent factor for coronary atherosclerosis.[11]

In cyanotic CHD, endothelial dysfunction predisposing to atherosclerosis is accompanied with anti-atherosclerotic thrombocytopenia, hyperbilirubinemia, hypercholesterolemia and hypotension.[4,5,12] Both invasive and non-invasive studies have shown that the incidence of coronary artery disease is reduced in adults with cyanotic CHD.[12,13] There has been no information regarding the risk factors of atherosclerosis in childhood in such patients.

The objective of the present study was to assess the systemic endothelial function and the risk for atherosclerosis in children with irreversible pulmonary hypertension due to cyanotic or acyanotic CHDs.

MATERIALS AND METHODS

This study was a case–control study. Informed consent was obtained from each patient included in the study. The study was initiated upon approval from the Hospital Ethics Committee. We recruited 18 patients with irreversible pulmonary hypertension due to CHD, who were followed at the Pediatric Cardiology Department, the Medical Faculty of Akdeniz University, and an age- and body mass index (BMI)-matched control group consisting of 18 children.

The patient group consisted of patients with ES who were diagnosed with irreversible pulmonary hypertension by the catheter laboratory their age ranged from 7–18 years. The control group included age- and BMI-matched patients with no hypertension, diabetes mellitus and obesity, but innocent heart murmur where cardiac disease was excluded.

Age, sex, height, weight, BMI and systolic blood pressure (SBP) and diastolic blood pressure (DBP) readings of all patients were recorded. Transcutaneous saturation was measured in both patients and controls after 5 min of rest using a standard transcutaneous pulse oximeter at the finger.

Complete blood count, total cholesterol (TC), high-density lipoprotein–cholesterol (HDL-C), low-density lipoprotein–cholesterol (LDL-C), very low-density lipoprotein–cholesterol (VLDL-C) and triglyceride (TG) levels were measured in both groups after 8 h of fasting. The samples were taken in the morning and evaluated on the same day. TC was analyzed using the enzymatic colorimetric method, HDL-C and LDL-C were analyzed by the homogenous colorimetric method and TG was analyzed using the colorimetric method. All analysis was evaluated using a Roche/Hitachi Cobas C analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Hemoglobin levels, hematocrit concentration and platelet count were obtained with automated electronic particle counters.

Detection of endothelial dysfunction

Before the procedure, patients were asked to abstain from substances such as caffeine and smoking cigarette, which are known to have a potential effect on the endothelial functions. The brachial artery was identified with palpation in the antecubital space and marked with a pen on the skin while the patients were fasting and in supine position. After 10 min of rest, the basal diameter of the brachial artery was determined in a quiet room maintained at a constant temperature. Then, the cuff of the sphygmomanometer was placed slightly above the brachial artery from where the previous measurement was taken, and inflated at least 30 mmHg above the SBP and held in this position for approximately 3 min. Then, the cuff of the sphygmomanometer was removed. The brachial artery diameter was measured at 60 s using a 12 MHz probe by the GE Healthcare Vivid 7 Pro echocardiography. The reading was recorded as the maximum diameter. The images were transferred to a digital media. The measurements of vascular diameter were manually performed over the R-wave synchronized with the electrocardiography wave. Flow-mediated dilatation (FMD) was calculated by dividing the change in arterial diameter to the basal arterial diameter (%), employing the images of the hyperemic phase.[14]

Measurement of carotid artery intima media thickness

The carotid intima media thickness (CIMT) was measured for each patient in a quiet room maintained at a constant temperature after 10 min of rest before ultrasonographic imaging. Ultrasonographic evaluations were performed using a 12 megahertz probe by the GE Healthcare Vivid 7 Pro echocardiography. Measurements were obtained while patients were in supine position, with the head slightly extended and turned opposite to the examined carotid artery. Images of the right and left carotid artery were obtained in pre-defined scanning positions. The images were focused on the posterior (far) walls of the carotid arteries. The images were computerized and analyzed. During analysis, measurements were obtained from the far wall because of the poor images from the proximal walls. Three measurements were carried out and their mean value was taken.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 for Windows program. Students' *t*-test was used when assumptions for the parametric tests were met, whereas Mann–Whitney U test was used when assumptions for the parametric tests were unmet. The values were defined as mean ± SD for the results, which were obtained using Student's *t*-test. The values were defined as median (minimum - maximum), which was obtained using the Mann–Whitney U test. *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Patients characteristics

The study included 18 patients (10 females, and eight males) with irreversible pulmonary hypertension due to CHD, who were followed at the Pediatric Cardiology Department, Medical Faculty of Akdeniz University, and an age- and BMI-matched control group consisting of 18 children (10 females and eight males) with innocent heart murmur. In the cyanotic patient group, there were seven complete atrioventricular septal defects, two large ventricular septal defects, one large ventricular septal defects and patent ductus arteriosus, three double-outlet right ventricles, two ventricular septal defects and transposition of the great arteries, two univentricular connection and single atrioventricular valve and one truncus arteriosus among patients. Four of those patients with atrioventricular septal defect also had Down's syndrome. The patients were on digoxin, furosemide and hydrochlorothiazide. Besides anticongestive threapy, six patients received bosentan and four patients received iloprost trometamol. The control group received no drugs.

When we compared the patient group and the control group, the mean transcutaneous oxygen saturation was lower in the patients than in the controls (*P*-value < 0.001). No statistically significant difference was observed between the groups in SBP and DBP values (*P*-value = 0.239 and 0.512, respectively) [Table 1].

Blood analysis

Factors that may have an impact on the endothelial function and CIMT were analyzed in both groups [Table 2]. In the cyanotic CHD group, hemoglobin and hematocrit concentrations were statistically significantly higher while platelet count was lower. The levels of TC, LDL-C and VLDL-C were statistically significantly lower compared to the control group (*P*-value = 0.001 and 0.006 and 0.014, respectively), whereas no statistically significant difference was found in the levels of HDL-C and TG (*P*-value = 0.113 and 0.975, respectively) [Table 2].

Results for measurements of brachial artery, FMD and CIMT

The comparison of both groups [Table 3] showed no statistically significant difference in the brachial artery basal diameter and the brachial artery diameter at 1 min (*P*-value = 0.963 and 0.669, respectively). No statistically significant difference was observed between the groups in CIMT (*P*-value = 0.299); however, FMD was statistically significantly reduced in the cyanotic CHD group (*P*-value < 0.001). ns-not significant

DISCUSSION

ES was described in 1897 by Victor Eisenmenger.[1,15] Later, Wood elucidated right-to-left shunt occurs as a result of elevated PAP and resistance in large cardiac defects.[16]

In ES and cyanotic CHD, chronic hypoxemia is associated with compensatory erythrocytosis, hyperviscosity and increased shear stress. An acute increase in shear stress causes vasodilation as a result of increase in endothelial nitric oxide production where endothelial function is maintained. Although there is an increase in the basal nitric oxide production in cyanotic CHDs, in which the increase in shear stress is continuous, there occurs vasoconstriction and endothelial dysfunction due to increased consumption and reduced nitric oxide bioavailability.[3] Nitric oxide plays a major role in the regulation of the cardiovascular system.[17] Endothelial and inducible nitric oxide synthase activities change in cardiac tissue from patients with cyanotic CHDs. While endothelial nitric oxide synthase activity and gene expression are downregulated, there is a compensatory increase in inducible nitric oxide synthase activity and gene expression in the macrophages and leukocytes.[18] Besides nitric oxide, oxygen also has a major role in the regulation of the vascular tone. In cyanotic

Table 1: Clinical characteristics of the patients and control groups

	Patient group	Control group	*P*-value
Age (years)	12.28 ± 3.26	11.78 ± 3.00	NS
BMI (kg/m^2)	17.82 ± 2.56	18.16 ± 1.87	NS
SBP (mmHg)	107.50 (85–115)	110.00 (95–115)	NS
DBP (mmHg)	62.28 ± 3.95	63.11 ± 3.66	NS
O_2 saturation	84.00 (76–88)	98.00 (95–100)	<0.001

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; O_2: Oxygen, NS: Not significant

Table 2: Complete blood count and lipid analysis results

	Patient group	Control group	*P*-value
HGB (g/dL)	16.51 ± 2.41	13.16 ± 1.11	<0.001
HCT (%)	50.45 ± 7.76	38.85 ± 3.22	<0.001
PLT count (×103)	207.50 (136–396)	311.50 (190–415)	<0.001
TC (mg/dL)	119.50 (97–158)	151.00 (127–196)	0.001
HDL-C (mg/dL)	48.00 (22–69)	52.00 (28–65)	NS
LDL-C (mg/dL)	59.50 (23–98)	74.00 (45–145)	0.006
VLDL-C (mg/dL)	20.83 ± 11.64	24.94 ± 19.07	0.014
TG (mg/dL)	88.00 (44–135)	81.50 (67–296)	NS

HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet; HDL-C: High-density lipoprotein–cholesterol; LDL-C: Low-density lipoprotein–cholesterol; VLDL-C: Very low-density lipoprotein–cholesterol; TG: Triglyceride, NS: Not significant

Table 3: Results for measurements of brachial artery, FMD and CIMT

	Patient group	Control group	*P*-value
BABD (mm)	2.82 ± 0.44	2.83 ± 0.41	NS
BAD at 1 min (mm)	3.03 ± 0.46	3.10 ± 0.47	NS
FMD (%)	5.26 ± 2.42	9.48 ± 2.60	<0.001
CIMT (mm)	0.41 ± 0.08	0.39 ± 0.06	NS

BABD: Brachial artery basal diameter; BAD: Brachial artery diameter; FMD: Flow-mediated dilation; CIMT: Carotid intima media thickness, NS: Not significant

CHDs, regulatory function of oxygen is also impaired because of hypoxemia.[19]

Endothelial dysfunction is a risk factor for atherosclerosis, and can be achieved by measuring venous occlusion-induced dilation of forearm vessels using plethysmography and FMD of the brachial artery using ultrasound.[20-22] The endothelial dysfunction invasively determined by FMD and the endothelial dysfunction in coronary circulation are similar.[23]

In the present study, we found systemic endothelial dysfunction with FMD in children with irreversible pulmonary hypertension due to CHDs. Conversely, Pedersen *et al.*[24] indicated that FMD is preserved in adults with cyanotic CHD. Different from the study by Pedersen *et al.*, we recruited children and included only cases of CHD with irreversible pulmonary hypertension in order to achieve homogeneity among patients in the cyanotic group. Therefore, this is the first study in children with irreversible pulmonary hypertension due to CHDs.

Perloff *et al.*[13] indicated that the risk for coronary atherosclerosis was not increased as assessed angiographically in the of adults with CHD, while Duffels *et al.*[12] found reduction in CIMT in patients with cyanotic CHD and no increased risk for atherosclerosis. They attributed this reduced risk for atherosclerosis to lower TC levels, thrombocytopenia, hyperbilirubinemia and lower SBP and DBP. Similarly, in a study on adult patients, Fyfe *et al.*[25] found that TC levels were low in cyanotic CHD and angiographically coronary arteries were dilated and atheroma free.

The present study found no increase in CIMT in children with irreversible pulmonary hypertension in CHDs. However, we found that the levels of TC, LDL-C and VLDL-C significantly decreased in the cyanotic group. There was no significant difference in the levels of HDL-C, TG and SBP and DBP when we compared the two groups. Although endothelial dysfunction in our patients, there was no evidence of increasing CIMT. These results may be due to a combination of atherosclerotic and anti-atherosclerotic factors in these patients. The long-term follow-up of these patients is important because the risk of atherosclerosis is not clear.

In conclusion, we found systemic endothelial dysfunction in children with irreversible pulmonary hypertension due to CHDs as assessed with FMD. However, we found no increased in the risk-factors for coronary atherosclerosis. The risk for atherosclerosis may depend on a balance between the systemic endothelial dysfunction with an atherosclerotic effect, polycythemia and the reduced TC levels with an anti-atherosclerotic effect anf lower platelet concentrations in children with irreversible pulmonary hypertension due to CHDs. Further studies are needed with a larger number of cases and a longer follow-up period to evaluate the same.

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