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## Vasoactive mediators in congenital heart diseases with shunt lesions and pulmonary hypertension

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**Aim:** Increased pulmonary flow in left-to-right shunt lesions and decreased pulmonary flow and hypoxia in right-to-left shunt lesions constitute the main cause of morbidity and mortality in congenital heart diseases. This study was planned to determine the relation of vasoactive mediators and pulmonary hypertension in congenital heart disease with shunt lesions.

**Materials and methods:** Patients (31 females, 22 males; 5 months to 16 years old) were divided into 2 groups according to their shunt lesions (acyanotic, cyanotic), pulmonary artery mean pressure (below 30 mmHg: nonhypertensive, above 30 mmHg: hypertensive), and pulmonary resistance (below 2 U/m<sup>2</sup>: low, above 2 U/m<sup>2</sup>: high). The acyanotic group was further divided into subgroups based on pulmonary artery mean pressure values. Vascular endothelial growth factor, interleukin 6, interleukin 8, fibroblast growth factor, and endothelin levels were determined in systemic arterial, pulmonary artery, and venous blood samples.

**Results:** All vascular endothelial growth factor levels were significantly elevated in the high pulmonary resistance group. Arterial and venous endothelin levels were significantly elevated in the cyanotic group and high pulmonary resistance group. Positive correlations were found between vascular endothelial growth factor and interleukin 6, interleukin 8, and pulmonary artery systolic pressures.

**Conclusion:** Vascular endothelial growth factors play an important role in the development of irreversible pulmonary hypertension; therefore, evaluation of vascular endothelial growth factor levels could guide the management of treatment.

**Key words:** Vascular endothelial growth factor, interleukin 6, interleukin 8, fibroblast growth factor, endothelin, congenital heart disease

### 1. Introduction

Incidence of congenital heart diseases (CHDs) is 5–8 per 1000 live births, and this is an important mortality and morbidity cause for children (1). Pulmonary vascular constitution and flow play an important role in the etiopathogenesis and clinical symptoms of CHDs. In acyanotic CHD with left-to-right shunt lesions pulmonary artery blood flow increases depending on the type and severity of the shunt, and the effect of increasing blood flow on the vascular endothelium causes pulmonary hypertension (2).

Cyanotic congenital heart diseases lead to hypoxia and a decrease in blood oxygen saturation because of right-to-left shunt. However, in some patients, despite tissue hypoxia there is not adequate development in the pulmonary vascular bed, which keeps patients from being totally treated, leading to increased morbidity and mortality (3).

The effect of increased pulmonary blood flow and hypoxia on patients shows variation. Differences in the release and interaction of mediators that regulate angiogenesis can be

responsible for different responses in patients and can affect the severity and distribution of diseases.

The aim of this study was to find the relation between pulmonary flow in CHD and the factors affecting the development of pulmonary vascular bed and the role of these factors in diagnosis and treatment.

### 2. Materials and methods

This study consisted of 53 patients (31 females, 22 males; mean age  $4.31 \pm 4.19$  years; range: 5 months to 16 years) who had diagnostic and therapeutic heart catheterization in the Department of Pediatric Cardiology. During heart catheterization, right atrium (RA), right ventricle (RV), pulmonary artery (PA), left atrium (LA), aorta (AO), and left ventricle (LV) pressure curves and blood samples were taken, and oxygen saturation measurements were done. From hemodynamic measurements, systemic flow (Qs), pulmonary blood flow (Qp), the ratio of flows (Qp/Qs), the amount of left-to-right and right-to-left shunt, pulmonary resistance (Rp), and systemic resistance (Rs) values were calculated according to Fick's principle.

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Patients were divided into 2 groups, acyanotic (Group 1; left-to-right shunt) and cyanotic (Group 2; right-to-left shunt). The acyanotic group was further divided into subgroups: with pulmonary hypertension and without pulmonary hypertension. In order to examine the effects of the angiogenic factors on the development of pulmonary hypertension and pulmonary vascular bed, patients were also grouped according to their pulmonary artery pressure and pulmonary resistance.

The patients with mean pulmonary artery pressure equal to or below 30 mmHg were assigned to the nonhypertensive group and the patients with mean pulmonary artery pressure over 30 mm Hg to the hypertensive group (4).

The patients whose pulmonary resistance was below  $2 \text{ U/m}^2$  were assigned to the low pulmonary resistance group and the patients whose pulmonary resistance was over  $2 \text{ U/m}^2$  to the high pulmonary resistance group.

The level of serum vascular endothelial growth factor (VEGF) was studied in 53 patients, and it was sampled from the pulmonary artery, systemic artery, and systemic vein.

The level of fibroblast growth factor, which was sampled from the systemic artery and systemic vein, was studied in 35 patients.

The levels of plasma interleukin 6 (IL-6), interleukin 8 (IL-8), and endothelin were sampled from the systemic artery and systemic vein in 43 patients.

The blood samples were centrifuged for 10 min at 3500 rpm at room temperature 30 min after drawing, and separated serums were stored at  $-70^\circ\text{C}$ .

Serum vascular endothelial growth factor, IL-6, and IL-8 were studied with BIOSOURCE International Immunoassay kits cat#8800 - KHGO112/KHGO111, cat#8800 - KHCOO62/KHCO061, and cat#8800 - KHCOO82/KHC0081, respectively. The level of endothelin was studied with BIOMEDICA equipment (cat#B1-20052). The level of fibroblast growth factor was studied with ELISA Oncogene (cat#8800; Q 1A67) immunoassay kits.

Vascular endothelial growth factor, IL-6, and IL-8 kits were provided within the scope of the research project SBAG-2483 by the Scientific and Technological Research Council of Turkey (TÜBİTAK).

The study protocol was approved by the Faculty of Medicine's ethics committee. Informed consent was obtained from all patients' families.

The statistical analysis was performed with Minitab 13.0. The results are given as mean  $\pm$  standard deviation (minimum value–maximum value).  $P < 0.05$  was accepted as significant. The Student t-test was applied. The correlation analyses were performed in all patients and for both the acyanotic and cyanotic groups separately.

### 3. Results

#### 3.1. Hemodynamic parameters

The mean and standard deviation values of hemodynamic parameters according to groups are shown in Table 1.

The mean and standard deviation values of hemodynamic parameters according to subgroups of the acyanotic group (Group 1) are shown in Table 2. Systemic and pulmonary flows, ratio of flows, amount of shunt, and systolic and

**Table 1.** Hemodynamic parameters of acyanotic and cyanotic groups (mean  $\pm$  SD).

Parameter	Acyanotic group (n = 36)	Cyanotic group (n = 17)	P
Hb (g/dL)	11.91 $\pm$ 1.89	15.01 $\pm$ 2.81	0.000
AO O <sub>2</sub> sat (%)	96.2 $\pm$ 2.19	82.47 $\pm$ 10.68	0.000
PA O <sub>2</sub> sat (%)	82.91 $\pm$ 7.72	81.93 $\pm$ 7.15	>0.05
Qp (L/min/m <sup>2</sup> )	10.2 $\pm$ 6.62	6.63 $\pm$ 4.55	>0.05
Qs (L/min/m <sup>2</sup> )	4.24 $\pm$ 1.20	4.83 $\pm$ 3.31	>0.05
Qp/Qs	2.43 $\pm$ 1.37	1.54 $\pm$ 0.95	0.044
Shunt (L/min/m <sup>2</sup> )	6.00 $\pm$ 6.19		
PAP sys (mmHg)	40.36 $\pm$ 22.75	36.5 $\pm$ 24.28	>0.05
PAP mean (mmHg)	28.22 $\pm$ 19.26	30.27 $\pm$ 20.43	>0.05
Rp (U/m <sup>2</sup> )	3.32 $\pm$ 5.75	3.02 $\pm$ 1.86	>0.05
LVSP (mmHg)	102.53 $\pm$ 16.63	93.76 $\pm$ 15.82	0.049
RVSP (mmHg)	54.28 $\pm$ 28.22	98.65 $\pm$ 16.67	0.000

AO: aorta, Hb: hemoglobin, LVSP: left ventricular systolic pressure, PA: pulmonary artery, PAP: pulmonary artery pressure, Qp: pulmonary flow, Qs: systemic flow, Rp: pulmonary resistance, RVSP: right ventricular systolic pressure, sat: saturation, sys: systolic.

**Table 2.** Hemodynamic values of acyanotic with pulmonary hypertension and groups without pulmonary hypertension (mean  $\pm$  SD).

Parameter	With pulmonary hypertension (n = 20)	Without pulmonary hypertension (n = 16)	P
Hb (g/dL)	11.08 $\pm$ 1.79	12.39 $\pm$ 1.81	0.044
AO O <sub>2</sub> sat (%)	95.67 $\pm$ 2.74	96.48 $\pm$ 1.86	>0.05
PA O <sub>2</sub> sat (%)	84.17 $\pm$ 8.36	82.26 $\pm$ 7.47	>0.05
QP (L/min/m <sup>2</sup> )	14.23 $\pm$ 9.02	7.92 $\pm$ 3.20	0.004
QS (L/min/m <sup>2</sup> )	4.79 $\pm$ 1.18	3.93 $\pm$ 1.12	0.038
QP/QS	2.96 $\pm$ 1.90	2.12 $\pm$ 0.86	>0.05
Shunt (L/min/m <sup>2</sup> )	9.57 $\pm$ 8.70	3.99 $\pm$ 2.83	0.007
PAP sys (mmHg)	63.00 $\pm$ 23.27	27.57 $\pm$ 7.01	0.000
PAP mean (mmHg)	47.15 $\pm$ 20.67	17.52 $\pm$ 5.04	0.000
Rp (U/m <sup>2</sup> )	6.14 $\pm$ 8.97	1.72 $\pm$ 1.15	0.024
LVSP (mmHg)	98.77 $\pm$ 12.21	104.65 $\pm$ 18.60	>0.05
RVSP (mmHg)	68.23 $\pm$ 23.94	46.39 $\pm$ 27.83	0.023

AO: aorta, Hb: hemoglobin, LVSP: left ventricular systolic pressure, PA: pulmonary artery, PAP: pulmonary artery pressure, Qp: pulmonary flow, Qs: systemic flow, Rp: pulmonary resistance, RVSP: right ventricular systolic pressure, sat: saturation, sys: systolic.

mean pulmonary artery pressures were significantly high in the acyanotic group with pulmonary hypertension.

### 3.2. Angiogenic factors

#### 3.2.1. Vascular endothelial growth factor

The levels of vascular endothelial growth factor of the acyanotic and cyanotic groups are given in Table 3.

There was not any statistical difference between the levels of vascular endothelial growth factor of the groups, although the level of vascular endothelial growth factor was higher in the acyanotic group.

The levels of vascular endothelial growth factor of the acyanotic subgroups, with pulmonary hypertension and without pulmonary hypertension, are given in Table 4.

There was not any statistical difference between the vascular endothelial growth factor levels of the subgroups of acyanotic group, although the level of vascular endothelial growth factor was high in the acyanotic group with pulmonary hypertension (Table 4).

The angiogenic factor levels according to pulmonary artery pressure are given in Table 5. In the hypertensive group (patients with high pulmonary artery pressure, whether cyanotic or acyanotic), the level of vascular endothelial growth factor was insignificantly high.

The angiogenic factor levels according to pulmonary artery resistance are given in Table 6. The vascular endothelial growth factor levels were significantly high in

the high pulmonary resistance group for arterial, venous, and pulmonary arterial blood samples.

#### 3.2.2. Endothelin

Endothelin levels of the cyanotic group (Table 3) and high pulmonary resistance group (Table 6) were significantly higher than in the acyanotic group and low pulmonary resistance group, respectively.

#### 3.2.3. Fibroblast growth factor

There were no significant differences between the fibroblast growth factor values of the acyanotic and cyanotic groups (Table 3) and the subgroups of acyanotic group (Table 4). Venous fibroblast growth factor values were significantly elevated in the hypertensive group (Table 5) and the high pulmonary resistance group (Table 6).

#### 3.2.4. Interleukin 6

Interleukin 6 values of the cyanotic (Table 3), acyanotic with pulmonary hypertension, (Table 4), hypertensive (Table 5), and low pulmonary resistance (Table 6) groups were high, but there were no significant differences between groups.

#### 3.2.5. Interleukin 8

Interleukin 8 values of acyanotic with pulmonary hypertension (Table 4), hypertensive (Table 5), and high pulmonary resistance (Table 6) groups were elevated, but only venous IL-8 levels of the high pulmonary resistance group were significantly high.

**Table 3.** Angiogenic factor levels of acyanotic and cyanotic groups (mean  $\pm$  SD).

Angiogenic factor (pg/mL)	Acyanotic, mean $\pm$ SD (n)	Cyanotic, mean $\pm$ SD (n)	P
VEGF (A)	190.6 <sup>a</sup> $\pm$ 26.3 (36)	83.6 <sup>a</sup> $\pm$ 05.0 (17)	>0.05
VEGF (V)	219.2 <sup>b</sup> $\pm$ 32.8 (36)	125.2 <sup>b</sup> $\pm$ 207.1 (17)	>0.05
VEGF (PA)	177.2 <sup>c</sup> $\pm$ 89.3 (36)	115.2 <sup>c</sup> $\pm$ 15.4 (15)	>0.05
FGF (A)	6.57 $\pm$ 3.01 (25)	7.40 $\pm$ 3.49 (10)	>0.05
FGF (V)	7.34 $\pm$ 3.42 (25)	6.83 $\pm$ 2.52 (10)	>0.05
Endothelin (A)	1.80 $\pm$ 3.36 (27)	4.80 $\pm$ 3.27 (16)	0.014
Endothelin (V)	1.89 $\pm$ 3.54 (27)	5.10 $\pm$ 3.38 (16)	0.016
IL-6 (A)	4.85 $\pm$ 7.90 (27)	32 $\pm$ 109.6 (16)	>0.05
IL-6 (V)	4.09 $\pm$ 6.12 (27)	38.3 $\pm$ 128.0 (16)	>0.05
IL-8 (A)	22.4 $\pm$ 67.1 (27)	26.4 $\pm$ 47.0 (16)	>0.05
IL-8 (V)	27.0 $\pm$ 68.4 (27)	44.1 $\pm$ 68.1 (16)	>0.05

<sup>a</sup>: Differences in systemic artery–systemic vein at <0.05, <sup>b</sup>: differences in systemic vein–pulmonary artery at <0.05, <sup>c</sup>: differences in systemic artery–pulmonary artery at <0.05.

VEGF (A): arterial vascular endothelial growth factor, VEGF (V): venous vascular endothelial growth factor, VEGF (PA): pulmonary arterial vascular endothelial growth factor, FGF (A): arterial fibroblast growth factor, FGF (V): venous fibroblast growth factor, endothelin (A): arterial endothelin, endothelin (V): venous endothelin, IL-6 (A): arterial interleukin 6, IL-6 (V): venous interleukin 6, IL-8 (A): arterial interleukin 8, IL-8 (V): venous interleukin 8.

**Table 4.** Angiogenic factor levels of acyanotic with pulmonary hypertension and acyanotic without pulmonary hypertension groups.

Angiogenic factor (pg/mL)	With pulmonary hypertension, mean $\pm$ SD (n)	Without pulmonary hypertension, mean $\pm$ SD (n)	P
VEGF (A)	265 $\pm$ 419 (20)	96 $\pm$ 91 (16)	>0.05
VEGF (V)	300 $\pm$ 425 (20)	117 $\pm$ 93 (16)	>0.05
FGF (A)	6.78 $\pm$ 2.91 (25)	6.25 $\pm$ 3.29 (10)	>0.05
FGF (V)	8.17 $\pm$ 2.87 (25)	6.08 $\pm$ 3.93 (10)	>0.05
Endothelin (A)	2.14 $\pm$ 3.42 (15)	1.38 $\pm$ 3.38 (12)	>0.05
Endothelin (V)	2.31 $\pm$ 3.71 (15)	1.35 $\pm$ 3.37 (12)	>0.05
IL-6 (A)	6.21 $\pm$ 10.32 (15)	3.15 $\pm$ 2.51 (12)	>0.05
IL-6 (V)	5.43 $\pm$ 8.02 (15)	2.41 $\pm$ 0.99 (12)	>0.05
IL-8 (A)	36.4 $\pm$ 88.8 (15)	5.0 $\pm$ 0.0 (12)	>0.05
IL-8 (V)	43.4 $\pm$ 89.5 (15)	6.42 $\pm$ 4.91 (12)	>0.05

VEGF (A): arterial vascular endothelial growth factor, VEGF (V): venous vascular endothelial growth factor, VEGF (PA): pulmonary arterial vascular endothelial growth factor, FGF (A): arterial fibroblast growth factor, FGF (V): venous fibroblast growth factor, endothelin (A): arterial endothelin, endothelin (V): venous endothelin, IL-6 (A): arterial interleukin 6, IL-6 (V): venous interleukin 6, IL-8 (A): arterial interleukin 8, IL-8 (V): venous interleukin 8.

**Table 5.** Angiogenic factor levels of nonhypertensive and hypertensive groups.

Angiogenic factor (pg/mL)	Nonhypertensive, mean $\pm$ SD (n)	Hypertensive, mean $\pm$ SD (n)	P
VEGF (A)	82.3 $\pm$ 107.9 (27)	262.1 $\pm$ 396.1 (22)	0.028
VEGF (V)	102.8 $\pm$ 128.9 (27)	320.8 $\pm$ 411.6 (22)	0.012
VEGF (PA)	87.2 $\pm$ 108.6 (25)	258.9 $\pm$ 373.3 (22)	0.033
FGF (A)	6.17 $\pm$ 2.82 (20)	7.65 $\pm$ 3.41 (15)	>0.05
FGF (V)	6.61 $\pm$ 3.14 (20)	7.97 $\pm$ 3.12 (15)	0.032
Endothelin (A)	1.50 $\pm$ 2.47 (22)	3.93 $\pm$ 3.94 (18)	0.022
Endothelin (V)	1.49 $\pm$ 2.68 (22)	4.32 $\pm$ 3.98 (18)	0.011
IL-6 (A)	22.5 $\pm$ 93.7 (22)	7.91 $\pm$ 10.91 (18)	>0.05
IL-6 (V)	25.0 $\pm$ 106.1 (22)	7.22 $\pm$ 9.90 (18)	>0.05
IL-8 (A)	7.72 $\pm$ 7.51 (22)	43.8 $\pm$ 89.3 (18)	>0.05
IL-8 (V)	14.91 $\pm$ 33.69 (22)	58.4 $\pm$ 92.4 (18)	0.047

VEGF (A): arterial vascular endothelial growth factor, VEGF (V): venous vascular endothelial growth factor, VEGF (PA): pulmonary arterial vascular endothelial growth factor, FGF (A): arterial fibroblast growth factor, FGF (V): venous fibroblast growth factor, endothelin (A): arterial endothelin, endothelin (V): venous endothelin, IL-6 (A): arterial interleukin 6, IL-6 (V): venous interleukin 6, IL-8 (A): arterial interleukin 8, IL-8 (V): venous interleukin 8.

**Table 6.** Angiogenic factor levels of low and high pulmonary resistance groups.

Angiogenic factor (pg/mL)	Low pulmonary resistance, mean $\pm$ SD (n)	High pulmonary resistance, mean $\pm$ SD (n)	P
VEGF (A)	82.3 $\pm$ 107.9 (27)	262.1 $\pm$ 396.1 (22)	0.028
VEGF (V)	102.8 $\pm$ 128.9 (27)	320.8 $\pm$ 411.6 (22)	0.012
VEGF (PA)	87.2 $\pm$ 108.6 (25)	258.9 $\pm$ 373.3 (22)	0.033
FGF (A)	6.17 $\pm$ 2.82 (20)	7.65 $\pm$ 3.41 (15)	>0.05
FGF (V)	6.61 $\pm$ 3.14 (20)	7.97 $\pm$ 3.12 (15)	0.032
Endothelin (A)	1.50 $\pm$ 2.47 (22)	3.93 $\pm$ 3.94 (18)	0.022
Endothelin (V)	1.49 $\pm$ 2.68 (22)	4.32 $\pm$ 3.98 (18)	0.011
IL-6 (A)	22.5 $\pm$ 93.7 (22)	7.91 $\pm$ 10.91 (18)	>0.05
IL-6 (V)	25.0 $\pm$ 106.1 (22)	7.22 $\pm$ 9.90 (18)	>0.05
IL-8 (A)	7.72 $\pm$ 7.51 (22)	43.8 $\pm$ 89.3 (18)	>0.05
IL-8 (V)	14.91 $\pm$ 33.69 (22)	58.4 $\pm$ 92.4 (18)	0.047

VEGF (A): arterial vascular endothelial growth factor, VEGF (V): venous vascular endothelial growth factor, VEGF (PA): pulmonary arterial vascular endothelial growth factor, FGF (A): arterial fibroblast growth factor, FGF (V): venous fibroblast growth factor, endothelin (A): arterial endothelin, endothelin (V): venous endothelin, IL-6 (A): arterial interleukin 6, IL-6 (V): venous interleukin 6, IL-8 (A): arterial interleukin 8, IL-8 (V): venous interleukin 8.

### 3.3. Correlation analyses

The results of the correlation analyses for relations among angiogenic factors, hemodynamic parameters, and blood sampling sites are given in Table 7 for all patients in general and for the acyanotic and cyanotic groups.

#### 3.3.1. All patients

##### 3.3.1.1. Vascular endothelial growth factor

Systemic arterial vascular endothelial growth factor levels showed significant correlation with pulmonary arterial systolic pressure ( $r$ : 0.349) and mean pressure

**Table 7.** Correlations with angiogenic factors and hemodynamic parameters.

Angiogenic factor	All patients	Acyanotic group (left-to-right shunt)	Cyanotic group (right-to-left shunt)
VEGF (A)	sPAP (r: 0.349)*	sPAP (r: 0.430)	
	mPAP (r: 0.336)	mPAP (r: 0.417)	
	IL-8 (A) (r: 0.734)**	IL-6 (A) (r: 0.546)	
	IL-8 (V) (r: 0.660)	IL-6 (V) (r: 0.645)	
		IL-8 (A) (r: 0.546)	
		IL-6 (V) (r: 0.645)	
		AO O <sub>2</sub> sat (r: -0.497)	
VEGF (V)	IL-8 (A) (r: 0.671)	sPAP (r: 0.429)	
	IL-8 (V) (r: 0.582)	mPAP (r: 0.413)	
		IL-6 (A) (r: 0.521)	
		IL-6 (V) (r: 0.625)	
		IL-8 (A) (r: 0.886)	
		IL-8 (V) (r: 0.964)	
		AO O <sub>2</sub> sat (r: -0.496)	
VEGF (PA)	IL-6 (A) (r: 0.377)	sPAP (r: 0.437)	
	IL-6 (V) (r: 0.380)	mPAP (r: 0.413)	
	IL-8 (A) (r: 0.669)		
	IL-8 (V) (r: 0.642)		
		IL-6 (A) (r: 0.518)	
		IL-6 (V) (r: 0.613)	
		IL-8 (A) (r: 0.888)	
		IL-8 (V) (r: 0.881)	
		AO O <sub>2</sub> sat (r: -0.513)	
IL-8 (A)	Rp (r: 0.595)	Rp (r: 0.616)	
	sPAP (r: 0.633)	sPAP (r: 0.553)	sPAP (r: 0.649)
	mPAP (r: 0.670)	mPAP (r: 0.648)	mPAP (r: 0.746)
	AO O <sub>2</sub> sat (r: -0.561)	AO O <sub>2</sub> sat (r: -0.561)	AO O <sub>2</sub> sat (r: -0.676)
	VEGF (A) (r: 0.734)		
	VEGF (V) (r: 0.671)		
	VEGF (PA) (r: 0.669)		
FGF (A) (r: 0.371)			
IL-8 (V)	Rp (r: 0.625)	Rp (r: 0.616)	
	sPAP (r: 0.586)	sPAP (r: 0.553)	
	mPAP (r: 0.663)	mPAP (r: 0.648)	
	RVSP (r: 0.422)	RVSP (r: 0.446)	
	VEGF (A) (r: 0.660)		
	VEGF (V) (r: 0.582)		
	VEGF (PA) (r: 0.642)	AO O <sub>2</sub> sat (r: -0.561)	
AO O <sub>2</sub> sat (r: -0.561)			
IL-6 (A)	VEGF (PA) (r: 0.377)	VEGF	Qp (r: 0.818)
IL-6 (V)	VEGF (PA) (r: 0.380)	VEGF	Qp (r: 0.810)
FGF (A)	sPAP (r: 0.347)		sPAP (r: 0.640)
			mPAP (r: 0.635)
	IL-8 (A) (r: 0.371)		Rp (r: 0.700)



Table 7. (Continued).

FGF (V)	Qs (r: 0.387)		mPAP (r: 0.670) Rs (r: 0.822)
Endothelin (A)	Qp (r: -0.349)	PA O <sub>2</sub> (r: -0.396)	
	PA O <sub>2</sub> (r: -0.415)		
Endothelin (V)	Qp (r: -0.333)		
	Qp/Qs (r: -0.346)	PA O <sub>2</sub> (r: -0.403)	
	LVSP (r: -0.364)		
	AO O <sub>2</sub> (r: -0.336)		
	PA O <sub>2</sub> (r: -0.355)		

VEGF (A): arterial vascular endothelial growth factor, VEGF (V): venous vascular endothelial growth factor, VEGF (PA): pulmonary arterial vascular endothelial growth factor, IL-8 (A): arterial interleukin 8, IL-8 (V): venous interleukin 8, IL-6 (A): arterial interleukin 6, IL-6 (V): venous interleukin 6, FGF (A): arterial fibroblast growth factor, FGF (V): venous fibroblast growth factor, endothelin (A): arterial endothelin, endothelin (V): venous endothelin, AO: aorta, LVSP: left ventricular systolic pressure, PA: pulmonary artery, mPAP: pulmonary artery mean pressure, sPAP: pulmonary artery systolic pressure, Qp: pulmonary flow, Qs: systemic flow, Rp: pulmonary resistance, Rs: systemic resistance, RVSP: right ventricular systolic pressure, sat: saturation.

\*: P < 0.05 for r, \*\*: P < 0.01 for bolded r.

(r: 0.336), and IL-8 (arterial r: 0.734, venous r: 0.660) levels. The systemic venous vascular endothelial growth factor levels also showed significant correlation with IL-8 (arterial r: 0.671, venous r: 0.582) levels. The pulmonary arterial vascular endothelial growth factor levels showed significant correlation with IL-8 (arterial r: 0.669, venous r: 0.642) and IL-6 (arterial r: 0.377, venous r: 0.380), respectively.

### 3.3.1.2. Endothelin

Negative correlation was found between systemic arterial endothelin and both pulmonary flow (r: -0.349) and pulmonary artery oxygen saturation (r: -0.415). There were also negative correlations between systemic venous endothelin and pulmonary blood flow (r: -0.333), flow ratio (r: -0.346), left ventricular end-systolic pressure (r: -0.364), systemic arterial (r: -0.336), and pulmonary arterial oxygen saturation (r: -0.355).

### 3.3.1.3. Fibroblast growth factor

Systemic arterial fibroblast growth factor levels showed significant correlation with pulmonary artery systolic pressure (r: 0.347) and arterial venous IL-8 levels (r: 0.371). Venous fibroblast growth factor levels showed significant correlation with systemic flow (r: 0.387).

### 3.3.1.4. Interleukin 6

Systemic arterial and venous IL-6 levels showed significant correlation with pulmonary arterial vascular endothelial growth factor (r: 0.377, r: 0.380, respectively).

### 3.3.1.5. Interleukin 8

Systemic arterial IL-8 levels showed significant correlation with pulmonary resistance (r: 0.595), pulmonary artery systolic pressure (r: 0.633) and mean pressure (r: 0.670),

VEGF (arterial r: 0.734, venous r: 0.671, pulmonary arterial r: 0.669), and fibroblast growth factor (r: 0.371) and negative correlation with aortic O<sub>2</sub> saturation (r: -0.561). Venous IL-8 levels showed significant correlation with pulmonary resistance (r: 0.625), pulmonary artery systolic pressure (r: 0.586) and mean pressure (r: 0.663), right ventricular systolic pressure (r: 0.442), and VEGF (arterial r: 0.660, venous r: 0.582, pulmonary arterial r: 0.642) and negative correlation with aortic O<sub>2</sub> saturation (r: -0.561).

### 3.3.2. Acyanotic group

The correlation analysis results for the acyanotic group are given in Table 7.

#### 3.3.2.1. Vascular endothelial growth factor

Significant correlation was found between systemic arterial, venous, and pulmonary artery VEGF levels and pulmonary artery systolic pressure (r: 0.430, r: 0.429, r: 0.437); pulmonary artery mean pressure (arterial VEGF r: 0.417, venous VEGF r: 0.413, pulmonary artery VEGF r: 0.413); arterial venous IL-6 (arterial VEGF r: 0.546, r: 0.645; venous VEGF r: 0.521, r: 0.625, pulmonary arterial VEGF r: 0.518, r: 0.613); and arterial venous IL-8 (arterial VEGF r: 0.546, r: 0.645; venous VEGF r: 0.886, r: 0.964, pulmonary arterial VEGF r: 0.888, r: 0.881) levels, respectively. The correlations with systemic arterial, venous, and pulmonary arterial VEGF levels and systemic arterial oxygen saturation were negative (r: -0.497, r: -0.496, r: -0.513).

#### 3.3.2.2. Interleukin 8

Significant correlation was found between both systemic arterial and venous IL-8 levels and pulmonary resistance (arterial r: 0.616, venous r: 0.616) and pulmonary artery



systolic pressures (arterial r: 0.553, venous r: 0.553) and mean pressures (arterial r: 0.648, venous r: 0.648); the correlations with systemic arterial oxygen saturation were negative (arterial r: -0.561, venous r: -0.561).

### 3.3.2.3. Endothelin

Systemic arterial (r: -0.396) and venous (r: -0.403) endothelin levels showed negative correlations with pulmonary arterial oxygen saturation.

### 3.3.3. Cyanotic group

The correlations between factor levels and hemodynamic values are given in Table 7.

#### 3.3.3.1. Vascular endothelial growth factor

There was no correlation between VEGF levels and other factors.

#### 3.3.3.2. Fibroblast growth factor

Positive correlations were found between systemic arterial fibroblast growth factor and pulmonary resistance (r: 0.700) and pulmonary artery systolic pressures (r: 0.640) and mean pressures (r: 0.635). There were positive correlations between systemic venous fibroblast growth factor and both systemic resistance (r: 0.822) and pulmonary artery mean pressure (r: 0.670).

#### 3.3.3.3. Interleukin 6

The correlations between systemic arterial (r: 0.818) and venous IL-6 (r: 0.810) and pulmonary flow were positive.

#### 3.3.3.4. Interleukin 8

Although there was a positive correlation between systemic arterial IL-8 and pulmonary artery systolic pressure (r: 0.649) and pulmonary artery mean pressure (r: 0.746), the correlation with systemic arterial oxygen saturation was negative (r: -0.676).

#### 3.3.3.5. Endothelin

There was no correlation between systemic and venous endothelin levels and any other parameters.

## 4. Discussion

Pulmonary vascular structure and flow patterns are important in determining the type of therapy and prognosis in CHD. Increased or decreased pulmonary flow in shunt lesions interacts with vascular circulating factors. Hypoxia is a potent stimulator for VEGF production. The high levels of serum VEGF in cyanotic CHD have been described in recent studies (5-9). However, we did not find any significant difference between the acyanotic (with left-to-right shunt lesions) and cyanotic (right-to-left shunt lesions) groups despite the high VEGF levels of the acyanotic group (Table 3). We think that the conflict between the literature and our results arises from patient characteristics. The patients included in our study mostly had increased pulmonary blood flow, such as double-outlet right ventricle and transposition of great arteries, in

contrast to the literature. The subgroups of the acyanotic group (left-to-right shunt lesions with pulmonary hypertension) had high VEGF levels. According to correlation analysis, serum VEGF levels showed positive correlation with pulmonary artery pressure. Based on these findings, all patients were divided into 2 groups according to pulmonary artery pressure; the group with pulmonary artery mean pressure over 30 mmHg had high VEGF levels. However, we did not find any significant differences in serum VEGF levels between the groups (Tables 4 and 5).

Pulmonary artery pressure increases with the effect of increased pulmonary blood flow in CHD with shunt lesions (10). Eventually, pulmonary vascular resistance increases due to structural changes in the vascular bed. Based on that knowledge we divided the patients into 2 groups, 1 with high pulmonary resistance (greater than 2 U/m<sup>2</sup>) and 1 with low resistance (below 2 U/m<sup>2</sup>). The level of serum VEGF was significantly higher in the group with pulmonary vascular resistance over 2 U/m<sup>2</sup> (Table 6). These findings led us to conclude that VEGF comes into the picture in further phases of pulmonary hypertension and plays a part in structural variations that become irreversible. Accordingly, the determination of VEGF levels may also be a guide for pulmonary hypertension follow-up (11-13), and inhibition of the VEGF signaling pathway may play a role in the treatment of pulmonary hypertension (14,15).

Interleukin 6 is a cytokine that plays an important part in the inflammatory process and is released as a response to tissue damage (16-18). As a proangiogenic factor, IL-6 induces the release of VEGF (19). There was a positive correlation between IL-6 and VEGF serum levels in our study, as in previous studies.

Although the serum IL-6 levels were higher in the cyanotic group and patients with pulmonary artery pressure, the differences between the groups were not statistically significant (Table 3). The highest IL-6 level in the low pulmonary resistance group was 442 ng/mL and was found in a patient with cyanotic CHD with increased pulmonary flow whose pulmonary resistance was 1.9 U/m<sup>2</sup>. The IL-6 level of the low pulmonary resistance group, excluding this patient, was 3.56 ng/mL; this level was lower than in the high pulmonary resistance group, but the difference was not significant. Even though there was positive correlation between the IL-6 and VEGF serum levels, we could not find any relation with IL-6 for either pulmonary arterial pressure or resistance.

Interleukin 8 is another cytokine that has effects like IL-6. Interleukin 8 is also effective in angiogenesis and secretion of VEGF (20,21). The serum levels of IL-8 were high in the acyanotic group with pulmonary hypertension and the hypertensive group (mean pulmonary artery

pressure over 30 mmHg); however, these differences were not statistically significant. Significantly high serum levels of IL-8 were found in the group whose pulmonary vascular resistance was higher than 2 U/m<sup>2</sup>. Interleukin 8 showed positive correlation with VEGF in all patients. These findings show that there is a close relation between IL-8 levels and pulmonary vascular changes.

The fibroblast growth factor is one of the first defining angiogenic factors. It is secreted by endothelial cells and mast cells and stored by the extra cellular matrix (22–24). We did not find any statistically significant difference in serum fibroblast growth factor levels between the acyanotic and cyanotic groups. There was also no correlation of serum fibroblast growth factor levels with either aortic or pulmonary oxygen saturation, which suggests that there is no relation between the serum fibroblast growth factor levels and hypoxia, in contrast to the literature (25,26).

The serum fibroblast growth factor was significantly high in the group with pulmonary artery mean pressure over 30 mmHg and pulmonary vascular resistance over 2 U/m<sup>2</sup>. Based on these findings, the fibroblast growth factor may have a role in the development of pulmonary hypertension.

Plasma endothelin levels were found to be significantly higher in the cyanotic group than in the acyanotic group in this study. A negative correlation was also found between pulmonary artery oxygen saturation and endothelin levels. These findings show that hypoxia is a strong stimulant for endothelin secretion (27–29).

Endothelin levels of the groups show the same characteristics as VEGF and IL-8. They were high in the

acyanotic with pulmonary hypertension and hypertensive groups (mean pulmonary artery pressure over 30 mmHg). Significantly high levels of endothelin were found in the group with pulmonary vascular resistance higher than 2 U/m<sup>2</sup>. The level of serum endothelin plays a part in the remodeling of the pulmonary vascular bed and has a role in the persistence of pulmonary hypertension (30–33). According to our results, the level of endothelin increases due to hypoxia in CHD, and it plays an important part in cell proliferation and the narrowing of pulmonary vascular lumen (34,35).

Although many factors are involved in developing pulmonary hypertension, such as IL-6, IL-8, VEGF, and fibroblast growth factor, we did not find any correlation between the endothelin and these factors. This result shows that the effect of endothelin on the pulmonary vascular bed has a different mechanism, and its effect is independent from other factors in CHD.

In conclusion, endothelin receptor blockers can be used in patients who have pulmonary hypertension in cyanotic CHD. Additionally, the VEGF, fibroblast growth factor, and interleukin receptor blockers may be used in patients with pulmonary hypertension in acyanotic congenital heart diseases with left-to-right shunt lesions (36).

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