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ORIGINAL ARTICLE

Vascular Endothelial Growth Factor in Children with Cyanotic and Acyanotic Congenital Heart Disease

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Objective Vascular endothelial growth factor is a potent stimulators of angiogenesis. Children with cyanotic congenital heart disease often experience the development of widespread formation of collateral blood vessels, which may represent a form of abnormal angiogenesis resulting in increased morbidity and mortality. We undertook the present study to determine whether children with cyanotic congenital heart disease haveelevated serum levels of vascular endothelial growth factor compared to children with acyanotic heart disease.

Methods Serum was obtained from 35 children with cyanotic congenital heart disease and 30 children with acyanotic heart disease. Vascular endothelial growth factor levels was measured in the serum of these patients by sandwich enzyme immunoassay.

Results Vascular endothelial growth factor was significantly elevated in children with cyanotic congenital heart disease compared to children with acyanotic heart disease (150.3±48.1 pg/ml vs. 85.4±18.7 pg/ml, respectively, $p<0.001$). In the cyanotic group, oxygen saturation (SaO_2) was negatively correlated with VEGF ($r=-0.631$, $p<0.001$) while haemoglobin was positively correlated ($r= 0.781$, $p=0.007$). No significant correlations were found in the acyanotic group.

Conclusions Children with cyanotic congenital heart disease have elevated systemic levels of vascular endothelial growth factor directly related to the degree of cyanosis (SaO_2 and haemoglobin levels). These findings suggest that the widespread formation of collateral vessels in these children may be mediated by vascular endothelial growth factor.

Keywords Vascular Endothelial Growth Factor, Congenital Heart Dissease
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INTRODUCTION

Children with cyanotic congenital heart disease may experience the development of abnormal vascular channels that become a source of significant morbidity. Abnormal vessel proliferation in these children may take several forms, including systemic-to-pulmonary collateral arteries (1–4), systemic-to-pulmonary venous collaterals (5), systemic venous collateral channels after bidirectional cavopulmonary anastomosis (6,7), and pulmonary arteriovenous malformations (8). It has been postulated that Vascular Endothelial Growth Factor (VEGF) may be responsible for the abnormal vessel proliferation. Several studies have demonstrated that VEGF expression is induced by hypoxia (9, 10, 11). Starnes and colleagues (12) reported that children with cyanotic congenital heart disease have elevated systemic levels of VEGF.

The purposes of this study was to determine whether children with cyanotic congenital heart disease have elevated serum levels of vascular endothelial growth factor (VEGF) compared to children with acyanotic heart diseases. Also, whether the VEGF levels correlates with the degree of cyanosis.

PATIENTS AND METHODS

From September 2007 to March 2008, 65 consecutive children were prospectively entered into the study. The children's age ranged from 10 months to 10.5 years; the children were divided into two groups those with cyanotic congenital heart disease (CHD) and those with acyanotic congenital heart diseases. All the patients were recruited from the Pediatric and Cardiology Departments of Cairo University Hospitals.

The first group consisted of 35 children (19 females, 16 males; age range: 10 months–9.8 years) with cyanotic CHD while the second group included 30 children (16 females, 14 males; aged between 12 months and 10.5 years) with acyanotic congenital heart disease.

Abbreviations and Acronyms
VEGF = Vascular Endothelial Growth Factor
BMI = Body Mass Index
CHD = Congenital Heart Disease
SaO_2 = Oxygen Saturation

All the children were subjected to the following:

- Full history and clinical examination, including the weight and height. The nutritional status of the patients was assessed by BMI [weight (kg)/height (m^2)].
- Labratory investigations: Full routine laboratory investigations were performed with special emphasis on:
  1. Haemoglobin concentration and haematocrit.
  2. Arterial oxygen satuation: Arterial oxygen saturation (SaO2) was analyzed in blood samples drawn from the peripheral vessels.
  3. Level of vascular endothelial growth factor (VEGF) in serum: Blood samples for VEGF analyses were withdrawn by standard venipuncture and were centrifuged for 10 minutes at 5000 rpm and then serum samples were stored at (-20°C) until the time of analysis. Serum VEGF levels was measured with sandwich enzyme immunoassay using commercially available kits, Human VEGF, Cytimmune Sciences Inc., Rockville, MD, USA).
- Chest X-ray: PA and lateral views.
- Twelve lead electrocardiogram.
- Echocardiography: M-mode and 2-dimensional examinations was performed from the standard subcostal, parasternal and apical approaches. Doppler (pulsed wave and continuous wave) and Colour-Doppler mapping was also used to reach the diagnosis.
- Cardiac catheterization: This was performed when definitive diagnosis could not be reached. Both right and left sided cardiac catheterization was performed and pressures were measured in all chambers and from the pulmonary artery and pulmonary veins (wedge pressure). Saturations in all the chambers was also performed to detect shunts.

Clinical diagnoses in both groups are listed in Table 1.

Table 1. Diagnoses in Cyanotic and Acyanotic Groups

Group	Diagnosis	Number of patients	Female/ Male
Cyanotic group I (n = 35)	Tetralogy of Fallot	20	(19/16)
	Double Outlet RV	6	
	TGA+VSD	7	
	PA+VSD	2	
Acyanotic group II (n= 30)	VSD	18	(16/14)
	ASD	8	
	PDA	4	

Exclusion criteria included:

- Patients with acute illness.
- Patients scheduled for surgery during the withdrawal of blood samples.
- Patients with pulmonary hypertension.

Data were analyzed by the Statistics Package for Social Sciences Statistical Software (SPSS) 11.0. All results were expressed as the mean value ± standard deviation. The Student T-test was used for comparisons between the two groups. The correlations between the groups were assessed by Pearson correlation. A value of $p<0.05$ was interpreted as indicating statistical significance.

RESULTS

As regards the general characteristics of both patients, no significant difference was found between the groups for age ($p=0.652$), body weight or height (Table 2).

Nutritional status of the two groups was assessed by body weight, height and BMI. BMI levels were within the normal range in the cyanotic group and acyanotic groups, showing absence of moderate or severe malnutrition in both groups; however, BMI levels were significantly lower in the cyanotic as compared to the acyanotic group ($p<0.001$) (Table 2).

Table 2. Demographic Data in Cyanotic and Acyanotic Groups

	Cyanotic	Acyanotic	P
Age (year)	3.1±1.6	3±1.7	0.652
Body weight (kg)	13.5±4.1	15.1±2.5	0.327
Height (cm)	93.1±14.6	95.2±12.1	0.738
BMI (kg/m2)	14.3±0.4	16.7±0.7	<0.001*

BMI: Body mass index.
Data are mean ± standard deviation.
* Indicates significant difference.

Serum VEGF was measured in all subjects (Table 3). The mean VEGF level was significantly higher in the cyanotic group as compared to the acyanotic group (150.3±48.1 pg/ml vs. 85.4±18.7 pg/ml, respectively, $p<0.001$).

Table 3. VEGF, Haemoglobin and Arterial Oxygen Saturation in Cyanotic and Acyanotic Groups.

	Cyanotic	Acyanotic	P
VEGF (pg/ml)	150.3±48.1	85.4±18.7	<0.001*
Haemoglobin (g/L)	13.5±1.7	11.81±0.97	<0.001*
SaO2 (%)	80.4±2.4	97.5±1.9	<0.001*

VEGF: Vascular endothelial growth factor. SaO2: Arterial oxygen saturation.
Data are mean ± standard deviation.
* Indicates significant difference.

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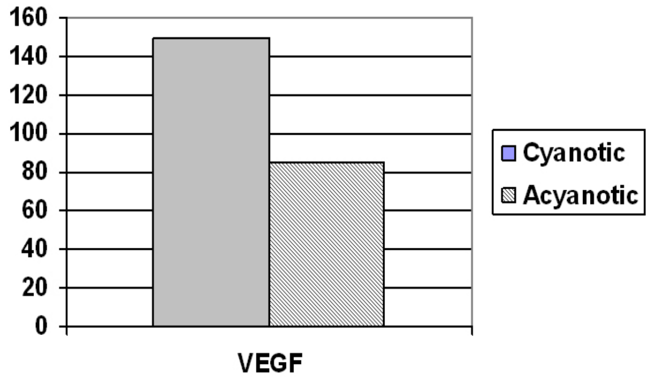

Figure 1: VEGF levels in cyanotic and acyanotic groups ($p < 0.001$).

SaO_2 values were significantly lower in the cyanotic group than those in the acyanotic group (80.4±2.4 and 97.5±1.9, respectively; $p<0.001$). The SaO_2 values were compared with the VEGF level in each group. In the cyanotic group, SaO_2 was negatively correlated with VEGF ($r=-0.631$, $p<0.001$), No significant correlations were noted between VEGF, SaO_2 in the acyanotic group (Table 4).
Haemoglobin levels were significantly different between the groups (13.5±1.7 and 11.81±0.97, respectively; $p<0.001$). Haemoglobin levels were compared with VEGF in each group. Haemoglobin was positively correlated ($r = 0.781$, $p=0.007$) with VEGF in the cyanotic group.

No correlations were noted between the VEGF, haemoglobin and SaO_2 levels in the acyanotic group (Table 4).

Table 4. Correlation Coefficients in Cyanotic and Acyanotic Groups

	Cyanotic		Acyanotic	
	VEGF		VEGF	
	R	P	R	P
Haemoglobin	0.781	0.007	0.27	0.217
SaO2 (%)	- 0.631	<0.001*	- 0.321	0.347

VEGF: Vascular endothelial growth factor. SaO2: Arterial oxygen saturation.
* Indicates significant correlation.

DISCUSSION

VEGF is a potent mitogen acting specifically on vascular endothelial cells, and is known to play a role in angiogenesis in widely divergent circumstances, such as embryonic development (13), wound healing (14), tumor growth (15), rheumatoid arthritis (16), and ischemic retinopathy (17). VEGF has been demonstrated to induce angiogenesis, endothelial cell proliferation, and migration, thereby promoting blood vessel growth. Recent studies have demonstrated that angiogenesis, facilitated by administration of angiogenic growth factors as in recombinant protein therapy or gene transfer, may be augmented in animal models of myocardial ischemia (18). Therapeutic angiogenesis with VEGF was recently performed in order to reduce the unfavorable tissue effects caused by ischemia (19). However, it was still unknown whether VEGF plays an important role in angiogenesis or abnormal vessel proliferation of the congenital heart disease in children.

The present study demonstrated that VEGF level was significantly elevated in children with cyanotic heart disease compared to children with acyanotic heart disease, these are similar to previous studies (12,20). It can be explained by hypoxia which is a strong stimulus for angiogenesis and leads to an upregulation of VEGF. No correlations were found, in the acyanotic group, between the VEFG level and the oxygen saturation or the haemoglobin level, suggesting that the main stimulus for VEGF elevation was the cyanosis present (low oxygen saturation and elevated haemoglobin levels).

LIMITATIONS

A limitation to this study was the broad variation in circulatory dynamics within the cyanotic group. The cyanotic group consisted of patients with various congenital heart diseases, such as tetralogy of Fallot causing decreased pulmonary blood flow, or a double outlet right ventricle causing increased pulmonary blood flow. Variability of the underlying hemodynamics and anatomy of the cyanotic group may make consistent analysis impossible. VEGF elevation may depend not only on systemic oxygen saturation but also on other factors, such as cytokines. More detailed studies are required to resolve this question.

Also, VEGF elevation may be related to the function of time, it was difficult for us to standardize an age limit for our cohort of patients (e.g. 2 year old children with CHD). Also the normal level of VEGF according to age is still uncertain (20).

Our study was also limited by the number of patients and the lack of visualization of the arteriovenous connections within the cyanotic group.

In Summary: The present study represents a preliminary attempt to identify factors that may have an impact on manifestations of cyanotic congenital heart disease. VEGF appears to be systemically elevated in patients with chronic cyanosis and may contribute to the formation of extensive collateral vessels that sometimes develop in these children. Issues related to the exact origin of these factors are not specifically answered by this study. However, these findings may have broader implications regarding the pathophysiologic features of cyanotic heart disease, while further study of affected children may aid in understanding the control mechanisms of angiogenesis.

CONCLUSION

In conclusion, children with cyanotic heart disease have elevated levels of VEGF (compared to children with acyanotic heart disease) and this elevation is directly correlated with the haemoglobin concentration and inversely correlated with the level of hypoxia. These findings suggest that the widespread formation of collateral vessels in these children may be mediated by vascular endothelial growth factor.

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