Vascular endothelial growth factor in children with cyanotic and acyanotic and congenital heart disease

Yasser Baghdady[1], Yasser Hussein[2], Mohamed Shehata[3]

[1]Cardiology Department, Faculty of Medicine, Cairo University, Egypt
[2]Paediatric Department, Faculty of Medicine, Cairo University, Egypt
[3]Clinical Pathology Departments, Faculty of Medicine, Cairo University, Egypt

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Corresponding author:
Ass. Prof. Yasser Baghdady
Cardiology Department
Faculty of Medicine
Cairo University, Egypt
E-mail:
yasserbaghdady@hotmail.com

Abstract

Introduction: Vascular endothelial growth factor is a potent stimulator 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 have elevated serum levels of vascular endothelial growth factor compared to children with acyanotic heart disease.
Material and methods: Serum was obtained from 35 children with cyanotic congenital heart disease and 30 children with acyanotic heart disease. Vascular endothelial growth factor levels were 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 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.

Key words: vascular endothelial growth factor, congenital heart disease.

Introduction

Angiogenesis, the formation of new capillary blood vessels, contributes to progression of a variety of diseases, such as tumour dissemination [1], rheumatoid arthritis [2, 3], and diabetic retinopathy [4, 5]. On the other hand, in conditions such as ischaemic cardiovascular disease [6, 7], ulcer healing [8, 9], and wound healing [10, 11], it is a physiological response to recover from organ injury because the restoration of blood flow is essential for oxygen and nutrient delivery to the healing site. Remarkable amounts of neovascularization develop in patients with cyanotic congenital heart disease who have low pulmonary blood flow and systemic cyanosis [12, 13]. The neovascularization in these patients has a compensatory role in systemic hypoxia and may be important for organ survival.

Abnormal vessel proliferation in these children may take several forms, including systemic-to-pulmonary collateral arteries [14-17], systemic-to-pulmonary venous collaterals [18], systemic venous collateral channels after bidirectional cavopulmonary anastomosis [19, 20], and pulmonary arteriovenous malformations [21]. 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 [22-24]. It has also been reported that children with cyanotic congenital heart disease have elevated systemic levels of VEGF [25]. These studies included a small number of patients and need to be validated.

We hypothesized that VEGF may mediate the abnormal angiogenesis which is seen in children with cyanotic congenital heart disease. Thus, the purpose of this study was to determine whether children with cyanotic congenital heart disease have elevated serum levels of VEGF compared to children with acyanotic heart diseases, and also whether the VEGF levels correlate with the degree of cyanosis.

Material and methods

From September 2007 to March 2008, 65 consecutive children were prospectively entered into the study. Oral consent was obtained from the parents of the children involved in 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 Paediatric 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.

All the children were subjected to the following:

- full history and clinical examination, including weight and height; the nutritional status of the patients was assessed by BMI [weight (kg)/height (m^2)];
- laboratory investigations:
  - haemoglobin concentration and haematocrit,
  - arterial oxygen saturation: arterial oxygen saturation (SaO_2) was analysed in blood samples drawn from the peripheral vessels,
  - level of VEGF in serum: blood samples for VEGF analyses were withdrawn by standard venipuncture and were centrifuged for 10 min at 5000 rpm and then serum samples were stored at –20°C until the time of analysis; serum VEGF level was measured with sandwich enzyme immunoassay using commercially available kits (Human VEGF, Cytimmune Sciences Inc., Rockville, MD, USA),
  - other investigations included kidney function, complete blood picture and prothrombin time and concentration;
- chest X-ray: PA and lateral views;
- twelve-lead electrocardiogram;
- echocardiography: using a Sonos 5500 ultrasound system (HP Hewlett Packard), with a 5 MHz transducer for children. M-mode and 2-dimensional examinations were performed from the standard subcostal, parasternal and apical approaches; Doppler (pulsed wave and continuous wave) and colour Doppler mapping were also used to reach the diagnosis;
- cardiac catheterization: using a Philips biplane cardiac catheterization laboratory:
  - this was performed in:
    - patients with pulmonary atresia: mainly to determine the origin and distribution of collaterals,
    - patients with tetralogy of Fallot: to define the coronary arteries and to determine the left ventricular diastolic volume and index; also to determine the origin and distribution of collaterals.

Both right and left sided cardiac catheterization was performed and pressure was measured in all chambers and from the pulmonary artery and pulmonary veins (wedge pressure). Saturation in all the chambers was also performed to detect shunts. Clinical diagnoses in both groups are listed in Table I.

Exclusion criteria included:

- patients with acute illness;
- patients who were critically ill, including those with moderate to severe malnutrition, according to the growth indices (Table II);
- patients scheduled for surgery during the withdrawal of blood samples;
- patients with pulmonary hypertension.

Data were analysed by the Statistics Package for Social Sciences Statistical Software (SPSS) 11.0. All results were expressed as the mean value ± standard deviation. Student's *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 patients, no significant difference was found between the groups for age ($p = 0.652$), body weight or height (Table II). Nutritional status of the two groups was assessed by body weight, height and BMI. Body mass index levels were within the normal range in

Table I. 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	

RV – right ventricle, TGA – transposition of great arteries, VSD – ventricular septal defect, PA – pulmonary atresia, ASD – atrial septal defect, PDA – patent ductus arteriosus

Table II. Indices used to determine the nutritional status of the children included in the study [26]

Nutritional status	Weight/age	Height/age	Weight/height	% IBW
Wasting	Normal or low	Normal	< 5th percentile	< 85-90%
Stunting	< 5th percentile	< 5th percentile	Normal	Normal
Mild malnutrition	Normal or low	Normal	< 5th percentile	81-90%
Moderate malnutrition	Normal or low	Normal	< 5th percentile	70-80%
Kwashiorkor	Normal or low	Normal or low	Normal (oedema)	Normal

IBW – ideal body weight

the cyanotic group and acyanotic group, 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 III).

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

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 correlation was noted between VEGF and SaO_2 in the acyanotic group (Table IV).

Table III. 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/m^2]	14.3 ±0.4	16.7 ±0.7	< 0.001*

BMI – body mass index, data are mean ± standard deviation
**Indicates significant difference*

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 V).

Discussion

The present study demonstrated that VEGF level was significantly elevated in children with cyanotic heart disease compared to children with acyanotic

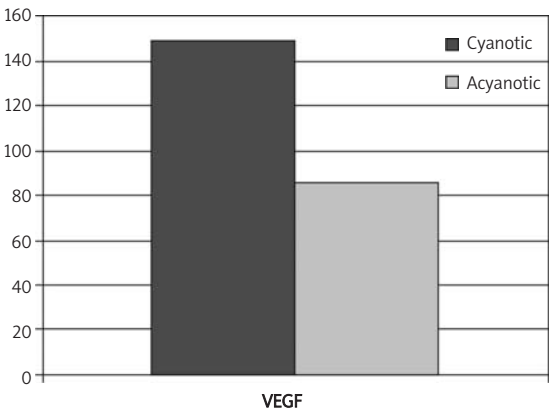

Figure 1. Vacular endothelial growth factor levels in cyanotic and acyanotic groups ($p < 0.001$)

Table IV. Vascular endothelial growth factor, haemoglobin and arterial oxygen saturation in cyanotic and acyanotic groups

	Cyanotic	Acyanotic	Value of *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*
SaO_2 [%]	80.4 ±2.4	97.5 ±1.9	< 0.001*

VEGF – vascular endothelial growth factor, SaO_2 – arterial oxygen saturation, data are mean ± standard deviation
**Indicates significant difference*

Table V. Correlation coefficients in cyanotic and acyanotic groups

	Cyanotic VEGF		Acyanotic VEGF	
	R	*p*	*R*	*p*
Haemoglobin	0.781	0.007	0.27	0.217
SaO_2 [%]	–0.631	< 0.001*	–0.321	0.347

VEGF – vascular endothelial growth factor, SaO_2 – arterial oxygen saturation
**Indicates significant correlation*

heart disease. These results are similar to previous studies [25, 32]. This elevated level of VEGF in the cyanotic group can be explained by the hypoxia, which is a strong stimulus for angiogenesis and leads to upregulation of VEGF. The lack of correlation, in the acyanotic group, between the VEGF level and the oxygen saturation or the haemoglobin level suggests that the main stimulus for VEGF elevation was the cyanosis present (low oxygen saturation and elevated haemoglobin levels).

The importance of neovascular formation in children with cyanotic heart disease is that it increases morbidity and mortality. An example of this is the development of aortopulmonary collateral arteries associated with pulmonary atresia. This may cause a number of problems, including significant left to right shunting, progressive obliteration after unifocalization procedures, and pulmonary "steal" from systemic blood flow during cardiopulmonary bypass. The management of children with cyanotic congenital heart disease may be complicated by the development of these vascular lesions and may require interventional cardiac catheterization or surgical treatment [25].

Vascular endothelial growth factor 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 [27], wound healing [9-11], tumour growth [28], rheumatoid arthritis [2, 3, 29], and ischaemic retinopathy [30]. Vascular endothelial growth factor 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 ischaemia [31]. Therapeutic angiogenesis with VEGF was recently performed in order to reduce the unfavourable tissue effects caused by ischaemia [32].

The present study represents a preliminary attempt to identify factors that may have an impact on manifestations of cyanotic congenital heart disease. Vascular endothelial growth factor 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 pathophysiological features of cyanotic heart disease, while further study of affected children may aid in understanding the control mechanisms of angiogenesis.

Limitations: a limitation of 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 haemodynamics and anatomy of the cyanotic group may make consistent analysis impossible. Vascular endothelial growth factor 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 [33].

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

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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