A Predictor of Collateral Formation in Congenital Heart Diseases

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Abstract

Objective: Vascular endothelial growth factor is 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.

The Aim of this 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.

Methods: Serum was obtained from 44 children with cyanotic congenital heart disease and 36 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 (159.3±48.1 pg/ml Vs. 85.4±18.7 pg/ml, respectively, p<0.001). In the cyanotic group, oxygen saturation (SaO₂) was negatively correlated with VEGF (r=-0.531, p<0.001) while hemoglobin was positively correlated (r=0.781, p=0.007). No significant correlations were found in the acyanotic group.

Conclusion: Children with cyanotic congenital heart disease have elevated systemic levels of vascular endothelial growth factor directly related to the degree of cyanosis (SaO₂ and hemoglobin 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 diseases.

Introduction

ANGIOGENESIS, the formation of new capillary blood vessels, contributes to a variety of disease progression such as tumour dissemination [1], rheumatoid arthritis [2,3] and diabetic retinopathy [4,5]. On the other hand, in conditions such as ischemic 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 arterio-venous 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 needed to be validated.

We hypothesized that vascular endothelial growth factor (VEGF) may mediate the abnormal angiogenesis, which is seen in children with cyanotic congenital heart disease. Thus, 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, 80 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 Pediatric and Cardiology Departments of Cairo University Hospitals.

The first group consisted of 44 children (24 females, 20 males; age range: 10 months-9.8 years) with cyanotic CHD while the second group included 36 children (19 females, 17 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 the weight and height. The nutritional status of the patients was assessed by BMI [weight (kg)/height (m²)].
- Laboratory investigations:
  - Hemoglobin concentration and haematocrit.
  - Arterial oxygen saturation: Arterial oxygen saturation (SaO₂) was analyzed in blood samples drawn from the peripheral vessels.
  - 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 5000rpm 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.
- Other investigations included the kidney functions, 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 5MHz transducer for children. 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: 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 pressures were measured in all chambers and from the pulmonary artery and pulmonary veins (wedge pressure). Saturations in all the chambers were also performed to detect shunts.

Diagnoses in both groups are listed in (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Number of patients</th>
<th>Female/Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanotic group I</td>
<td>Tetralogy of Fallot</td>
<td>24</td>
<td>(24/20)</td>
</tr>
<tr>
<td>(n=44)</td>
<td>Double Outlet RV</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGA+VSD</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PA+VSD</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Acyanotic group II</td>
<td>VSD</td>
<td>22</td>
<td>(19/17)</td>
</tr>
<tr>
<td>(n=36)</td>
<td>ASD</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDA</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>


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 2).
- Patients scheduled for surgery during the withdrawal of blood samples.
- Patients with pulmonary hypertension.

Statistical analysis:

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.
Table (2): Indices used to determine the nutritional status of the children included in the study [26].

<table>
<thead>
<tr>
<th>Nutrition Status</th>
<th>Weight/Age</th>
<th>Height/Age</th>
<th>Weight/Height</th>
<th>% IBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wasting</td>
<td>Normal or low</td>
<td>Normal</td>
<td>&lt;5th percentile</td>
<td>&lt;85-90%</td>
</tr>
<tr>
<td>Stunting</td>
<td>&lt;5th percentile</td>
<td>&lt;5th percentile</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mild malnutrition</td>
<td>Normal or low</td>
<td>Normal</td>
<td>&lt;5th percentile</td>
<td>81-90%</td>
</tr>
<tr>
<td>Moderate malnutrition</td>
<td>Normal or low</td>
<td>Normal</td>
<td>&lt;5th percentile</td>
<td>70-80%</td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>Normal or low</td>
<td>Normal or low</td>
<td>Normal (edema)</td>
<td>Normal</td>
</tr>
</tbody>
</table>

IBW = Ideal body weight.

Results

As regards the general characteristics of both patients, no significant difference was found between the groups for age (p=0.452), body weight or height (p=0.237) or (p=0.736). 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 3).

Table (3): Demographic data in cyanotic and acyanotic groups.

<table>
<thead>
<tr>
<th></th>
<th>Cyanotic</th>
<th>Acyanotic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>3.1±1.6</td>
<td>3±1.7</td>
<td>0.452</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>13.5±4.1</td>
<td>15.1±2.5</td>
<td>0.237</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>93.1±14.6</td>
<td>95.2±12.1</td>
<td>0.738</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>14.3±0.4</td>
<td>16.7±0.7</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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

Serum VEGF was measured in all patients (Table 4). The mean VEGF level was significantly higher in the cyanotic group as compared to the acyanotic group (159.3±48.1pg/ml Vs. 85.4±18.7pg/ml, respectively, p<0.001) (Fig. 1).

Table (4): VEGF, haemoglobin and arterial oxygen saturation in cyanotic and acyanotic groups.

<table>
<thead>
<tr>
<th></th>
<th>Cyanotic</th>
<th>Acyanotic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/ml)</td>
<td>159.3±48.1</td>
<td>85.4±18.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>14.5±1.7</td>
<td>11.8±0.97</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>80.4±2.4</td>
<td>97.5±1.9</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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

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

SaO₂ 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). (Fig. 2).

Fig. (2): O₂ saturations in cyanotic and acyanotic groups (p<0.001).
The \( \text{SaO}_2 \) values were compared with the VEGF level in each group. In the cyanotic group, \( \text{SaO}_2 \) was negatively correlated with VEGF (\( r = -0.531, p < 0.001 \)) (Fig. 3). No significant correlations were noted between VEGF, \( \text{SaO}_2 \) in the acyanotic group (Table 5).

Table (5): Correlation coefficients in cyanotic and acyanotic groups.

<table>
<thead>
<tr>
<th></th>
<th>Cyanotic VEGF</th>
<th>Acyanotic VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>0.781 0.007</td>
<td>0.27 0.217</td>
</tr>
<tr>
<td>( \text{SaO}_2 ) (%)</td>
<td>-0.531 &lt;0.001*</td>
<td>-0.321 0.347</td>
</tr>
</tbody>
</table>

| VEGF: Vascular endothelial growth factor. |
| \( \text{SaO}_2 \): Arterial oxygen saturation. |
| * Indicates significant correlation. |

Hemoglobin was positively correlated (\( r = 0.781, p = 0.007 \)) with VEGF in the cyanotic group (Fig. 4). No correlations were noted between the VEGF, hemoglobin and \( \text{SaO}_2 \) levels in the acyanotic group (Table 5).

**Discussion**

The present study demonstrated that VEGF level was significantly elevated in children with cyanotic heart disease compared to children with acyanotic 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 an upregulation of VEGF. The lack of correlations, in the acyanotic group, between the VEGF level and the oxygen saturation or the hemoglobin level, suggests that the main stimulus for VEGF elevation was the cyanosis present (low oxygen saturation and elevated hemoglobin levels). The importance of neovascular formation in children with cyanotic heart disease is important in 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 with increase volume overload to the ventricle, 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]. While pulmonary A-V collateral or systemic venous or venoarterial bring desaturated blood back to the heart so increase the cyanosis [18,21].

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 [27], wound healing [9-11], tumor growth [28], rheumatoid arthritis [2,3,29] and ischemic retinopathy [30]. 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 [31]. Therapeutic angiogenesis with VEGF was recently performed in order to reduce the unfavorable tissue effects caused by ischemia [32].
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 pathophysiology features of cyanotic heart disease, while further study of affected children may aid in understanding the control mechanisms of angiogenesis.

**Limitations:** In this study blood sampling from a healthy or non critically ill patients or without acute illness as a control was not feasible. 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. Transposition of the great arteries with ventricular septal defect causing increased pulmonary blood flow. Variability of the underlying hemodynamic 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.

Normal level of VEGF according to age is still uncertain [38].

Our study was also limited by the number of patients and the lack of visualization of the arteriovenous connections within the cyanotic group so we were unable to correlate the high level of vascular endothelial growth factor within the cyanotic patients with the presence or extent of collaterals.

Study is limited also as we are in need to follow-up our cyanotic patients postoperatively to study the effect of palliative or corrective surgery upon the VEGF level is will differ by the plan of management used.

**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 hemoglobin 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.

**Recommendation:**

Further studies are recommended to correlate between VEGF Level in cyanotic cardiac patients with the presence and extent of collaterals to use this VEGF in patient’s selection for invasive maneuvers, especially postoperatively as persistence elevation or re elevation of the VEGF postoperatively might affect the morbidity and may be helpful for selection of the patients who in need for cardiac catheterization postoperatively.

**Acknowledgements:**

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**References**


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