Correlation between vascular endothelial growth factor and leptin in children with cyanotic congenital heart disease

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SUMMARY: Aydın Hİ, Yozgat Y, Demirkaya E, Olgun A, Okutan V, Lenk MK, Özcan O. Correlation between vascular endothelial growth factor and leptin in children with cyanotic congenital heart disease. Turk J Pediatr 2007; 49: 360-364.

The objective in this study was to determine whether there was any relation between leptin and vascular endothelial growth factor (VEGF) in children with cyanotic and acyanotic heart anomalies. The study group consisted of 18 children with cyanotic congenital heart disease (CHD) and 20 age-adjusted children with acyanotic CHD as controls. Serum VEGF and leptin levels were determined by enzyme-linked immunosorbent assay (ELISA). The mean VEGF level was 149.25±42.93 pg/ml (range 80.66-217.00) in the cyanotic group and 88.18±20.94 pg/ml (range 48.44-112.71) in the acyanotic group ($p<0.001$). The mean leptin level was 7.55±1.46 ng/ml (range 4.08-10.25) in the cyanotic group and 6.89±1.43 ng/ml (range 2.67-8.57) in the acyanotic group ($p=0.168$). There was a significant positive correlation ($r=0.723$, $p<0.001$) between VEGF and leptin levels in the cyanotic group while there was no correlation ($r=0.235$, $p=0.348$) in the acyanotic group. Arterial oxygen saturation (SaO_2) was negatively correlated ($r= -0.625$, $p<0.001$) with VEGF, but not correlated with leptin ($r= -0.207$, $p=0.211$) in the cyanotic group. There was no correlation between VEGF, leptin and SaO_2 in the acyanotic group. We conclude that it is likely that both VEGF and leptin have a role in the pathogenesis of angiogenesis in cyanotic CHD.

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

It is well known that there is a remarkable neovascularization in patients with cyanotic congenital heart disease (CHD) who have low pulmonary blood flow and systemic cyanosis. Angiogenesis, the formation of new capillary blood vessels, in these patients appears to be linked to a compensatory response in systemic hypoxia[1]. Vascular endothelial growth factor (VEGF), which is necessary to initiate the formation of immature vessels by either vasculogenesis or angiogenic sprouting in the adult and during development, is known to be upregulated by hypoxia[2-4]. Elevated levels of serum VEGF have been documented in patients with cyanotic CHD and it has been pointed out that VEGF contributes to angiogenesis in patients with cyanotic CHD[4-6]. Recent studies have addressed the possibility that leptin, which was regarded as a circulating hormone primarily produced in adipocytes and responsible for regulation of energy balance through effects on the hypothalamus, may either induce angiogenesis or potentiate VEGF-mediated angiogenesis[1,7]. It has also been reported that there are similar leptin concentrations in children with cyanotic and acyanotic CHD despite significantly lower body mass index (BMI) in cyanotic children than in acyanotic children[8]. The relationship between VEGF and leptin in cyanotic CHD has not been determined to date even though there are known interactions between them. The aim of the present study was to compare plasma VEGF and leptin levels in patients with cyanotic versus acyanotic heart disease.

Material and Methods

Two groups of patients with cyanotic and acyanotic CHD being followed in the Department of Pediatrics between January 2001 and October 2005 were enrolled in the study. The study was approved and supported by the institutional ethical committee. Written informed consents were obtained from their parents. The first group consisted of 18 children (10 females, 8 males; aged 12 months–9.8 years) with cyanotic CHD and the second group included 20 children (11 females, 9 males; aged 13 months–10.1 years) with acyanotic CHD. All were diagnosed by clinical evaluation and laboratory investigations including chest X-ray, electrocardiography, echocardiography and cardiac catheterization. Clinical diagnoses in both groups are listed in Table I.

Table I. Specific Diagnoses in Cyanotic and Acyanotic Groups

Diagnosis	n (f/m)	%
Acyanotic group	20 (11/9)	55.5
Ventricular septal defect (VSD)	14 (8/6)	36.8
Atrial septal defect	6 (3/3)	15.7
Cyanotic group	18 (10/8)	44.5
Tetralogy of Fallot	13 (7/6)	34.2
Transposition of great arteries	3 (2/1)	7.8
Pulmonary atresia + VSD	2 (1/1)	5.2
Total	38	100

None of the patients had been operated nor had evidence of acute illness during the withdrawal of blood samples. Arterial oxygen saturation (SaO_2) was analyzed promptly in blood samples drawn from the radial artery. Blood samples for VEGF and leptin analyses were withdrawn by standard venipuncture after three hours of fasting 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 and leptin levels were measured with sandwich enzyme immunoassay using commercially available kits (Human Leptin ELISA, BioVendor Laboratory Medicine, Modrice, Czech Republic; CytELISA™, Human VEGF, Cytimmune Sciences Inc., Rockville, MD, USA, respectively). The nutritional status of the patients was assessed by BMI [weight (kg)/height (m^2)] as a measure of weight for height.

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 Mann-Whitney U 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

No significant difference was found between the groups for age and sex ($p=0.641$ and $p=0.529$; respectively) (Table II).

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, showing absence of moderate or severe malnutrition in this group; however, BMI levels were significantly lower in the cyanotic as compared to the acyanotic group ($p<0.001$) (Table II).

Table II. Demographic Data in Cyanotic and Acyanotic Groups

	Cyanotic	Acyanotic	p
Age (year)	3.2 ± 1.9	2.9 ± 1.9	0.641
Body weight (kg)	13.1 ± 5.3	14.7 ± 4.6	0.335
Height (cm)	92.5 ± 16.5	94.8 ± 13.1	0.642
BMI (kg/m^2)	14.9 ± 0.6	16.0 ± 0.9	<0.001*

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

Serum VEGF and leptin levels were measured in all subjects (Table III). The mean VEGF level was significantly higher in the cyanotic group as compared to the acyanotic group (149.25±42.93 pg/ml vs. 88.18±20.94 pg/ml, respectively, $p<0.001$). The mean leptin level was 7.55±1.46 ng/ml in the cyanotic group and 6.89±1.43 ng/ml in acyanotic group. There was no significant difference between the two groups for serum leptin levels ($p=0.168$). However, plasma leptin levels corrected for BMI were significantly higher in the cyanotic group as compared to the acyanotic group (0.51±0.10 vs. 0.42±0.08, respectively; $p=0.010$). Plasma leptin levels were correlated with BMI in the acyanotic group ($r=0.515$, $p<0.001$), but not in the cyanotic group ($r=0.418$, $p=0.093$).

Table III. Leptin, Leptin/BMI, VEGF, Hemoglobin and Arterial Oxygen Saturation in Cyanotic and Acyanotic Groups

	Cyanotic	Acyanotic	p
Leptin (ng/ml)	7.55 ± 1.46	6.89 ± 1.43	0.168
Leptin/BMI	0.51 ± 0.10	0.42 ± 0.08	0.010*
VEGF (pg/ml)	149.25 ± 42.93	88.18 ± 20.94	<0.001*
Hemoglobin (g/L)	13.87 ± 1.28	12.03 ± 0.82	<0.001*
SaO_2 (%)	82.6 ± 3.5	95.2 ± 2.8	<0.001*

BMI: Body mass index. VEGF: Vascular endothelial growth factor. SaO_2: Arterial oxygen saturation.
Data are mean ± standard deviation.
*Indicates significant difference.

When serum leptin and VEGF levels of the groups were compared (Fig. 1), a significant positive correlation was noted between the VEGF and leptin levels in the cyanotic group (r=0.723, p<0.001), while there was no correlation in the acyanotic group (r=0.235, p=0.348) (Table IV).

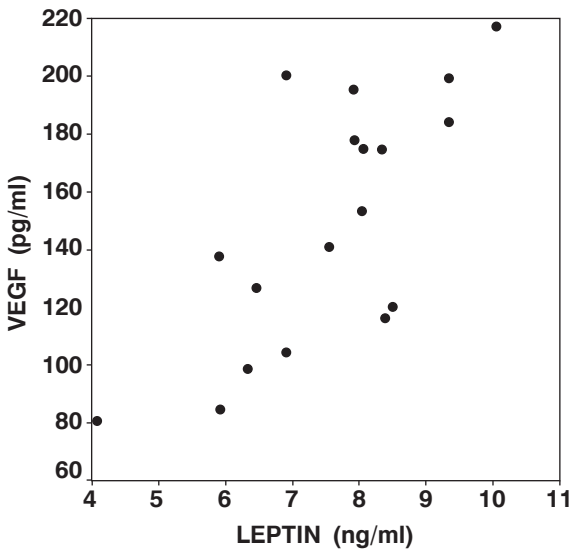

Fig. 1. Correlation of serum leptin with serum VEGF in the cyanotic group. Serum leptin positively correlated with serum VEGF (r=0.723, p<0.001).

SaO_2values were significantly lower in the cyanotic group than those in the acyanotic group (82.6±3.5 and 95.2±2.8, respectively; p<0.001). The SaO_2 values were compared with the VEGF and leptin levels in each group. In the cyanotic group, SaO_2 was negatively correlated with VEGF (r=-0.625, p<0.001), but not with leptin (r= -0.207, p=0.211). No significant correlations were noted between VEGF, leptin and SaO_2 in the acyanotic group (Table IV).

Hemoglobin levels were significantly different between the groups (13.87±1.28 and 12.03±0.82, respectively; p<0.001). Hemoglobin levels were compared with VEGF and leptin in each group. Hemoglobin was positively correlated (r= 0.553, p=0.009) with VEGF, but not correlated with leptin (r= 0.117, p=0.642) in the cyanotic group. There was also a significant negative correlation (r=-0.733, p<0.001) between hemoglobin and SaO_2 in the cyanotic group. No correlations were noted between the VEGF, leptin, hemoglobin and SaO_2 levels in the acyanotic group (Table IV).

Table IV. Correlation Coefficients in Cyanotic and Acyanotic Groups

	Cyanotic				Acyanotic			
	Leptin		VEGF		Leptin		VEGF	
	r	p	r	p	r	p	r	p
Leptin								
VEGF	0.723	<0.001*			0.235	0.348		
SaO_2	-0.207	0.211	-0.625	<0.001*	-0.057	0.810	-0.226	0.339
Hemoglobin	0.117	0.642	0.553	0.009*	0.096	0.687	0.290	0.215

VEGF: Vascular endothelial growth factor. SaO_2: Arterial oxygen saturation.
*Indicates significant correlation.

Discussion

Cyanotic CHD is often associated with various types of collateral vessels which were thought to be caused by several angiogenic factors such as VEGF, angioproteins, ephrins, or matrix metalloproteins. Among these angiogenic factors, VEGF has been known to promote the formation of collateral vessels in ischemic heart disease and cyanotic CHD[9-11]. Several studies have also demonstrated that tissue leptin and VEGF expression are similarly regulated by hypoxia through hypoxia-induced factor-1 transcriptional pathway[1,12]. In this study, we evaluated serum levels of VEGF and leptin in children with cyanotic and acyanotic CHD in order to address whether the presence of hypoxia influences systemic levels of angiogenic factors. VEGF levels in the cyanotic group were shown to be significantly higher than in the acyanotic control group. This finding was reported previously in experimental animal models and *in vivo* human studies[4,11]. We also demonstrated that VEGF levels were significantly negatively correlated with the severity of hypoxia in cyanotic patients. Himeno et al.[6] firstly showed that the elevated VEGF levels in children with cyanotic CHD aged 3 months to 10 years were negatively correlated with SaO_2 and positively correlated with hemoglobin; however, they did not observe these findings in cyanotic patients older than 10 years of age. The age range of our subjects was 1 to 10 years. We also found a positive correlation between serum VEGF and hemoglobin levels in the cyanotic group, but not in the acyanotic group, as previously reported by Himeno et al.[6]. In our study, we also found a significant negative correlation between hemoglobin and SaO_2. Gidding et al.[13] reported a significant negative correlation between hemoglobin and SaO_2 in children with cyanotic CHD aged 2-11 years, which was consistent with our findings. In the present study, we did not evaluate the parameters such as iron stores and erythropoietin levels that would be important in determining hemoglobin concentrations in cyanotic heart disease. Our findings are consistent with those in previous studies that support the interaction of hypoxia with VEGF in patients with cyanotic CHD.

Plasma leptin levels in children with cyanotic CHD were first studied by Hallioglu et al[8]. They investigated the correlations of leptin with nutritional and growth parameters and showed that cyanotic patients had significantly lower standard deviation scores of weight, mid-arm circumference, and left arm triceps skinfold thickness as compared to the acyanotic group, whereas leptin levels were similar in both groups. We observed that serum leptin concentrations were higher in the cyanotic subjects who had relatively lower BMI scores than in acyanotic subjects, but this difference did not reach statistical significance ($p=0.168$). However, when serum leptin levels were corrected for BMI (leptin/BMI), the leptin/BMI ratios were significantly higher in the cyanotic group than in the acyanotic group ($p=0.010$). It is well known that circulating leptin is positively correlated with BMI in humans[14,15]. These findings suggest the presence of some other additional factors that may contribute to the elevation of leptin in patients with cyanotic CHD. We suggest that hypoxia may be a contributing factor for higher levels of leptin despite lower BMI in children with cyanotic CHD. In our study, there was a negative correlation between serum leptin levels and SaO_2 but this was not statistically significant. This can be explained by lower fat stores which are the main source of leptin in these patients[14,15]. Guerre-Millo et al.[16] demonstrated that hypoxia directly increases leptin gene expression, leptin promoter activity, and leptin secretion in human adipose cells. Our findings are supported by reports of many in vivo and in vitro experiments showing that leptin is a hypoxia-inducible gene[16-18].

In addition to the increased VEGF and leptin concentrations in patients with cyanotic CHD, another noteworthy finding in our study is the presence of a positive correlation between leptin and VEGF in these patients ($r=0.723$, $p<0.001$). Suda et al.[4], Himeno et al.[6], and Hamada et al.[11] suggested that increased VEGF could contribute to active neovascularization in patients with cyanotic CHD; Park et al.[19] showed that leptin increased endothelial cell VEGF secretion in a dose-dependent manner. In the present study, we demonstrated a positive correlation between serum leptin and VEGF concentrations in patients with cyanotic CHD. Our findings of a significantly positive correlation between leptin and VEGF levels and a significantly negative correlation between VEGF and SaO_2 in children with

cyanotic CHD indicate that oxygen supply is a factor in the regulation of VEGF production and leptin may contribute to this process. However, our results do not allow us to demonstrate the exact relationship between leptin and hypoxia in patients with cyanotic CHD. Large-scale investigations of other hypoxia-inducible angiogenetic growth factors involved in different steps of angiogenesis are required to determine the underlying specific mechanism of action in cyanotic CHD.

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