

Study of Vascular Endothelial Growth Factor and Endostatin I N Childhood Leukemia and Lymphoma

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ABSTRACT

Background and purpose: Angiogenesis is an important component in the progression and metastasis of solid tumors. Angiogenesis is also critically involved in the pathogenesis of hematologic malignancies. Current data suggest important prognostic and therapeutic implications of angiogenesis in a variety of malignancies of the hematopoietic system, including acute and chronic leukemias, myeloproliferative diseases, multiple myeloma, non-Hodgkin's lymphomas and Hodgkin's disease. This study aims at clarifying the role of angiogenic factors, vascular endothelial growth factor (VEGF) and endostatin in the pathogenesis of leukaemia and lymphoma in children and to assess if these factors can be used as a prognostic factor for leukaemia and lymphoma.

Materials and methods: The present study examined the levels of VEGF and endostatin using ELISA technique in 40 cases, 20 cases presenting with acute leukemia (13 with ALL and 7 with AML) and 20 cases presenting with lymphoma (13 with NHL and 7 with HD).

Results: Serum levels of VEGF and endostatin were significantly higher at diagnosis and after complete remission than controls. Serum levels of VEGF have significant correlation with platelets, WBCs, Hb concentration and endostatin at diagnosis in cases of leukemia and lymphoma, but have no significant correlation with age. Serum levels of endostatin have significant correlations with Hb concentration and VEGF, but have no significant correlation with age, platelets count and WBCs. No significant differences could be found in serum VEGF and endostatin in different types of leukemia and lymphoma and patients with organomegaly or without organomegaly.

Conclusions: These data suggest that VEGF and endostatin expression ascribes an important role in children with leukemia and lymphoma. They may have a prognostic role for follow up of cases with leukemia and lymphoma.

Key Words: *Vascular endothelial growth factor (VEGF) - Endostatin - Angiogenesis - Childhood leukaemia - Childhood lymphoma.*

INTRODUCTION

Angiogenesis is an important component in the progression and metastasis of solid tumors. Angiogenesis is also critically involved in the pathogenesis of hematologic malignancies. Current data suggest important prognostic and therapeutic implications of angiogenesis in a variety of malignancies of the hematopoietic system, including acute and chronic leukemias, myeloproliferative diseases, multiple myeloma, non-Hodgkin's lymphomas and Hodgkin's disease [1].

The endothelial cell proliferation and microvessel formation are regulated by a wide range of soluble mediators, including angiogenin, angiopoietin-2, basic fibroblast growth factors, vascular endothelial growth factor (VEGF), VEGF-D, angiostatin and endostatin [2].

Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis by acting as a potent inducer of vascular permeability as well as serving as a specific endothelial cell mitogen. The importance of angiogenic factors such as VEGF, although clearly established in solid tumors, has not been fully elucidated in human hematopoietic neoplasms [3].

Endostatin, a C-terminal fragment of collagen XVIII, is an endogenous angiogenesis inhibitor. While endostatin is being investigated for its usefulness in treating solid tumors, its significance in hematologic malignancies is still unknown [4].

This study aims at clarifying the role of

angiogenic factors, vascular endothelial factor and endostatin in the pathogenesis of leukemia and lymphoma in children, and to assess if these factors can be used as a prognostic factor for leukaemia and lymphoma.

PATIENTS AND METHODS

We conducted our study on 40 cases, 20 leukemia and 20 lymphoma patients, admitted to Minia Center of Oncology during the period from January to October 2002. Leukemia cases included 13 patients with acute lymphoblastic leukemia and 7 with acute myeloid leukemia. Lymphoma cases comprised 7 Hodgkin's and 13 non-Hodgkin's lymphoma patients. Our patients were 26 males and 14 females, with ages ranging from 2 to 13 years. Sixteen [16] healthy age- and sex-matched children were included as controls.

Full clinical history was taken from all cases and controls. Patients were clinically examined for the presence of pallor, bone pain and/or limping, bleeding tendency (purpura, epistaxis, scleral hemorrhage), infection, hepatosplenomegaly, lymph node enlargement, testicular enlargement, gingival infiltration and any palpable masses.

Cases were also subjected to the following investigations:

- Complete blood picture
- Chest X-ray for any hilar lymph node enlargement
- Abdominal sonography
- Bone marrow examination
- Biopsy from enlarged lymph nodes or any accessible masses
- Cut Tomography Scanning for head, chest and abdomen
- Serum VEGF and endostatin which were measured by ELISA

Venous blood samples (5 ml) were collected from each case as well as from controls. After blood clotting, samples were centrifuged, sera separated, and stored at -70°C until the time of performing the assay to measure the level of VEGF and endostatin.

Statistical analysis:

Statistical analysis were performed using the software SPSS Inc. paired t-test were used for

correlations, where independent t-test were used to compare serum VEGF and endostatin concentrations in different groups. $p < 0.05$ is significant.

RESULTS

The present study included 40 patients who were admitted to Minia Center of Oncology from January to October 2002, 20 patients suffering from acute leukemia and 20 patients suffering from lymphoma. All cases were subjected to full clinical examination and laboratory investigation in addition to measuring the levels of serum VEGF and endostatin by using ELISA.

The results of this study revealed that there was a significant increase of serum levels of VEGF and endostatin in cases with leukemia and lymphoma than those of controls. Serum levels of VEGF decreased significantly after complete remission than those at diagnosis, but was still significantly higher than controls (Tables 1,2).

Serum levels of VEGF were correlated with platelet counts, WBCs, Hb concentration, age and endostatin at diagnosis in cases of leukemia and lymphoma. Correlation of VEGF with platelets, Hb, WBCs and endostatin was statistically significant, but had no significant correlation with age (Table 3). At the same time serum levels of endostatin had significant correlation with Hb concentration and VEGF, but had no significant correlation with age, platelet counts and WBCs (Table 4).

Also, this study revealed that serum levels of VEGF and endostatin at diagnosis increased significantly in non survivors than survivors in both leukemia and lymphoma cases (Table 5), but there were no significant differences in serum levels of VEGF and endostatin in different types of leukemia and lymphoma (Fig. 1) and patients with or without organomegaly (Fig. 2).

DISCUSSION

During the past few years, it has been proposed that angiogenesis may play a role not only in solid tumors, but also in hematopoietic malignancies. Previous studies of hematopoietic malignancies have demonstrated a close relationship between the bone marrow and angiogenesis [5].

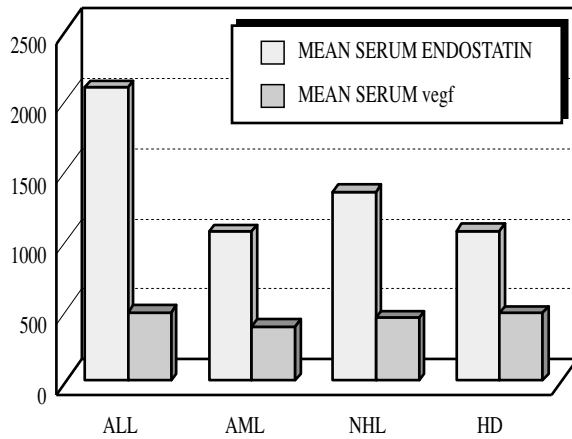


Fig. (1): Comparison of serum VEGF and endostatin in cases with leukemia (ALL and AML) and lymphoma (NHL and HD) at diagnosis.

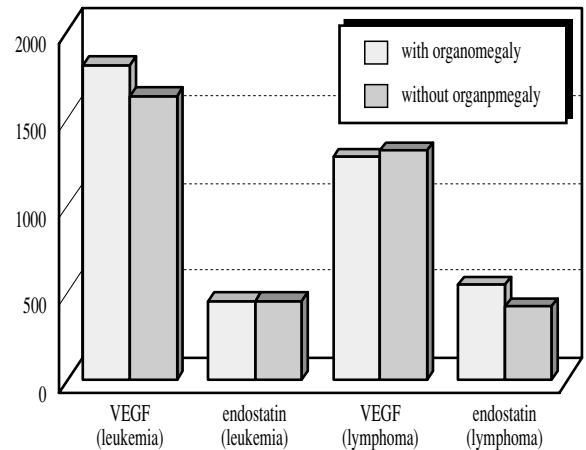


Fig. (2): Comparison of serum VEGF and endostatin in cases with leukemia and lymphoma with or without organomegaly at diagnosis.

Table (1): Statistical analysis of serum VEGF (p g/ml) in leukemia and lymphoma at diagnosis, after complete remission (CR), as well as in controls.

		N	Mean ± S.D	p value
Leukemia	At diagnosis (a)	20	1718.00 ± 1151.27	p ₁ 0.0001*
	After CR (b)	15	292.46 ± 145.20	p ₂ 0.0001*
	Controls (c)	16	73.12 ± 58.65	p ₃ 0.0001*
Lymphoma	At diagnosis (a)	20	1293.75 ± 949.37	p ₁ 0.0001*
	After CR (b)	16	316.25 ± 229.63	p ₂ 0.0001*
	Controls (c)	16	73.12 ± 58.65	p ₃ 0.001*

p₁ (a) vs (c) * p values ≤ 0.05 are considered significant
 p₂ (b) vs (c) p₃ (a) vs (b)

Table (2): Statistical analysis of serum endostatin (ng/ml) in leukemia and lymphoma at diagnosis, after CR, as well as in controls.

		N	Mean ± S.D	p value
Leukemia	At diagnosis (a)	20	449.20 ± 108.23	p ₁ 0.0001*
	After CR (b)	15	664.33 ± 196.33	p ₂ 0.0001*
	Controls (c)	16	139.31 ± 127.84	p ₃ 0.96
Lymphoma	At diagnosis (a)	20	468.00 ± 142.60	p ₁ 0.0001*
	After CR (b)	16	706.56 ± 177.20	p ₂ 0.0001*
	Controls (c)	16	139.31 ± 127.84	p ₃ 0.27

p₁ (a) vs (c) * p values ≤ 0.05 are considered significant
 p₂ (b) vs (c) p₃ (a) vs (b)

Table (3): Correlation of serum VEGF (pg/ml) in leukemia and lymphoma at diagnosis with age, hemoglobin concentration (Hb), platelets, white blood cell count (WBCs) and endostatin.

Variables correlated	Leukemia		Lymphoma	
	r	p	r	p
Serum VEGF and age	0.310	0.18	0.070	0.768
Serum VEGF and Hb	-0.802	0.0001*	-0.796	0.0001*
Serum VEGF and platelets	0.769	0.0001*	0.460	0.04*
Serum VEGF and WBCs	0.589	0.006*	0.602	0.005*
Serum VEGF and endostatin	0.796	0.0001*	0.689	0.001*

* p values ≤ 0.05 are considered significant

Table (4): Correlation of serum endostatin (ng/ml) in leukemia and lymphoma at diagnosis with age, hemoglobin concentration (Hb), platelets, white blood cell count (WBCs) and endostatin.

Variables correlated	Leukemia		Lymphoma	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Serum endostatin and age	0.286	0.22	0.263	0.62
Serum endostatin and Hb	-0.540	0.01*	-0.603	0.005*
Serum endostatin and platelet	0.341	0.41	0.390	0.08
Serum endostatin and WBCs	0.225	0.24	0.244	0.29
Serum endostatin and VEGF	0.796	0.0001*	0.689	0.001*

* *p* values ≤ 0.05 are considered significant.

Table (5): Statistical analysis of serum VEGF (*p* g/ml) and endostatin (ng/ml) in children with leukemia and lymphoma in relation to survival.

		Survivors		Non survivors		<i>p</i> values
		N	Mean \pm S.D.	N	Mean \pm S.D.	
Leukemia	VEGF	15	1258.66 \pm 654.99	5	3096.00 \pm 1276.51	0.0001*
	Endostatin	15	402.93 \pm 88.82	5	588.00 \pm 88.99	0.001*
Lymphoma	VEGF	16	960.93 \pm 480.36	4	2625.00 \pm 1265.24	0.0001*
	Endostatin	16	432.18 \pm 99.34	4	611.25 \pm 212.69	0.02*

* *p* values ≤ 0.05 are considered significant.

Several studies suggest that, like solid tumors, hematologic malignancies progress together with an induction of angiogenesis. Little is known about angiogenesis and angiogenesis-related molecules in leukemia. The normal vascular bed in the bone marrow forms a sinusoidal network supporting the hematopoietic cells, similar to cellular support in other organs such as kidney and spleen. Perez-Atayde and colleagues [6] found a significantly higher bone marrow microvessel density in ALL in comparison to controls. They also reported increased vascularity in 20 bone marrow samples from patients with acute myeloid leukemia (AML). Expression of VEGF in leukemic cells of patients with AML was also found by Fiedler and colleagues [7] and Hussong and coworkers [5].

In the present study, we evaluated VEGF and endostatin expression in newly diagnosed childhood leukemias and lymphomas, as well as, after complete remission. Our results showed that VEGF levels were significantly higher in newly diagnosed cases of leukemia than controls ($p < 0.0001$). These results are in agreement with Aguayo et al. [8] and Di Raimondo et al. [9], who reported that significantly elevated levels of VEGF are observed in a variety of hematological malignancies.

Bone marrow biopsy samples taken from

children with leukemia show significantly higher microvessel density than those from controls, suggesting that leukemic cells induce angiogenesis in the bone marrow and that leukemia might be angiogenesis dependent [6].

There were highly significant differences between serum VEGF levels in leukemic cases after complete remission and those in controls ($p < 0.0001$), but the levels of serum VEGF at diagnosis were significantly higher than those after complete remission ($p < 0.0001$). This may be attributed to the effect of chemotherapy on the bone marrow and improvement of hypoxia caused by anemia. These results are in harmony with those of Glenjen et al. [10] who reported that intensive chemotherapy could modulate the systemic component of angiogenic regulation in AML patients.

In the current study, high serum VEGF levels were strongly associated with a high leukocyte count and a high thrombocyte count ($p < 0.0001$ for both) in leukemic cases at diagnosis. These results coincide with those of Salven et al. [11], who suggested that high serum VEGF was strongly associated with a high leukocyte count and a high thrombocyte count ($p < .0001$). In addition, Aguaya et al. [8] reported that overall VEGF serum levels correlated with platelet and total white blood cells.

Data show that most, if not all, VEGF in the circulation is found in blood cells, including platelets and leukocytes, indicating that the VEGF detected in serum samples is released from the blood cells during the coagulation process [12]. However, even when the leukocyte and platelet counts are taken into account, the levels of circulating VEGF are generally higher in cancer patients than in healthy persons and leukocytes and platelets isolated from cancer patients contain highly elevated amounts of VEGF per blood cell [13]. High levels of VEGF have been reported in platelets, and it is possible that during the clotting process and the separation of the serum, VEGF is released from the platelets and WBC leading to the detection of high levels of such cytokine [14].

There was a significant negative correlation of serum VEGF levels ($p < 0.0001$) in relation to hemoglobin concentration, These results can be explained by hypoxia present due to anemia in leukemic patients, which stimulates the release of VEGF. Exposure to hypoxia induces VEGF expression rapidly [15]. Hypoxic upregulation of VEGF thus provides a compensatory mechanism by which tissues (or tumors) can increase their oxygenation through induction of blood vessel growth [16].

In the present study, there is a significant increase of serum VEGF levels in children with lymphoma when compared to controls ($p < 0.0001$). These results are in concordance with those of Salven et al. [11] who reported that serum VEGF concentrations in NHL patients are higher than controls. Also Giles (1) reported that in HD and NHL patients, there were statistically significant increases in VEGF as compared with normal controls.

In non-Hodgkin lymphomas, significantly higher microvessel counts have been found in high-grade lymphomas than in low-grade lymphomas, implying that angiogenesis in NHL increases with tumor malignancy grade [17]. In addition, NHL has been found to express angiogenic molecules, including VEGF and VEGF-C [18].

There was a significant decrease of serum VEGF levels in children with lymphoma after complete remission than at diagnosis ($p < 0.0001$), but these levels remained higher than controls. This is in agreement with the results of Giles

(1) who reported that in post-therapy samples from patients with HD, VEGF remained higher than controls.

In the present work, we found no significant association between the levels of serum VEGF and the type of leukemia (ALL and AML) and lymphoma (NHL and HD), ($p > 0.05$ and $p > 0.7$, respectively). This is in concordance with Salven et al. [11], Giles (1) and Koomagil et al. [19] who reported that no significant differences were found in serum VEGF levels among different types of leukemia and lymphoma. This is in disagreement with Aguayo et al. [8] who found that VEGF plasma levels were significantly increased in acute myeloid leukemia, but were insignificantly increased in acute lymphoblastic leukemia.

Regarding age, we found that there were no significant differences in the serum levels of VEGF in newly diagnosed leukemia or lymphoma cases ($p > 0.07$ and $p > 0.7$, respectively). These results are in agreement with Koomagil et al. [19], who reported that no significant relationship could be detected between VEGF and age in patients with ALL. Also, our results are in concordance with Salven et al. [11] who reported no significant associations between pre-treatment serum VEGF levels and age at diagnosis in cases with NHL.

In this work, there was no significant relation between serum VEGF levels and the extramedullary extension of disease (organomegaly) in cases with leukemia ($p > 0.9$) and lymphoma ($p = 0.2$) at diagnosis. These results are in harmony with Koomagil et al. [19] who reported insignificant differences between serum VEGF and extramedullary extension in patients with ALL. Our results are also in concordance with Salven et al. [11] who reported insignificant differences between serum VEGF and the number of extranodal tumor sites in cases with NHL. In the current study, there was higher serum VEGF levels at diagnosis in non survivors than those in survivors. These differences in serum levels of VEGF between survivors and non survivors in patients with childhood leukemia and lymphoma are highly significant ($p < 0.0001$ for both). These results are in agreement with Aguayo et al. [20], who estimated VEGF in patients with AML and Salven et al. [11] who measured VEGF in patients with NHL. Our results are also in agreement with Bertolini

et al. [21] who suggested that β -FGF and particularly VEGF, might be considered prognostic factors in NHL. At the same time, our results are in agreement with Bono et al. [22], who reported that patients who had high pretreatment levels of serum VEGF had particularly poor outcomes. On the other hand, our results are in disagreement with those of Giles (1), who suggested that the levels of these angiogenic factors did not correlate with survival in NHL and in HD.

Salven et al. [11,23] found that patients with high serum VEGF concentrations at diagnosis had inferior overall survival rates than those with lower serum concentrations of VEGF. High serum VEGF levels were also associated with poor prognosis in patients with NHL. Aguayo et al. [20] also found that elevated levels of VEGF were associated with reduced survival in patients with AML.

High serum concentrations of serum VEGF levels in patients with cancer are associated with several unfavourable clinical parameters. These include short tumor volume doubling time [24], progressive disease [25], extensive disease [26], and shorter survival [27].

Elevated levels of angiogenic growth factors are also associated with an adverse prognosis in patients with non-Hodgkin's lymphoma. In a single-institution study of 200 patients, levels of serum VEGF negatively correlated with prognosis [11].

In our study, there were highly significant differences in serum endostatin levels between leukemic patients at diagnosis and controls ($p < 0.0001$). This is in agreement with Glenjen et al. [10] who suggested that patients with untreated AML had increased levels of endostatin than controls.

In the current study, there were highly significant differences between serum endostatin levels in cases with lymphoma than controls. These results are in agreement with Bono et al. [22] who reported that serum endostatin levels were higher in patients with NHL compared with the control group.

A circulating form of human endostatin has been identified [28]. Intriguingly, the concentrations of soluble endostatin found in the serum

samples of healthy human donors [29] are similar to the concentrations that efficiently inhibit endothelial cell proliferation in vitro [30]. The circulating forms of endostatin may be involved in the homeostatic control of angiogenesis. Hence, it might be possible to obtain an angiogenic profile of a cancer patient's blood sample by measuring the concentrations of several circulating angiogenic and antiangiogenic molecules. This angiogenic profile might be used to monitor cancer therapy, or it might be a predictor of outcome after cancer has been diagnosed and even aid in the selection of the proper antiangiogenic treatment [11].

There were insignificant differences between serum levels of endostatin at diagnosis and after complete remission in cases of leukemia and lymphoma ($p > 0.9$ and $p > 0.2$, respectively).

In the present study, we found that no significant relationship existed between serum endostatin and platelet count ($p > 0.4$ and 0.08), or WBCs ($p > 0.2$ for both) in cases with leukemia and lymphoma. However, there was a significant negative correlation between serum endostatin and hemoglobin concentration in either leukemia or lymphoma cases ($p = 0.01$ and 0.005 , respectively). The negative correlation between serum endostatin and hemoglobin concentration may be explained by the indirect effect of hypoxia.

Regarding types of leukemia (ALL and AML) and lymphoma (NHL and HD) in our study, we found insignificant differences between serum endostatin levels and each of leukemia and lymphoma ($p > 0.06$ and 0.6 , respectively) at diagnosis.

In this work, there was no significant relationship between serum endostatin and the extramedullary extension of disease (organomegaly) in cases with leukemia ($p = 0.2$) and lymphoma ($p = 0.06$).

Our study revealed that there were significant high serum levels of endostatin in patients with leukemia at diagnosis in non survivors than in survivors ($p = 0.001$). This coincides with the results of Lai et al. [4] who suggested that, by multivariate analysis, plasma endostatin was found to be a significant predictor of overall survival ($p = 0.03$). The mechanism underlying

the association between high plasma endostatin and poor clinical outcome is unclear, although it may be related to the possible plasma endostatin reflection of tumor burden.

In the current study, we found that serum levels of endostatin in non survivors were higher than those in survivors in patients with lymphoma and these differences showed statistical significance ($p = 0.02$). This is in agreement with Bono et al. [22] who suggested that high pre-treatment levels of serum endostatin are associated with poor survival in patients with NHL.

As regards age relations with endostatin serum levels in cases with leukemia and lymphoma, there were no significant differences ($p > 0.2$ and $p > 0.6$, respectively).

In our study, we found a significant positive correlation between serum VEGF and serum endostatin at diagnosis in both patients with leukemia and lymphoma. This is in concordance with the results of Bono et al. [22], who reported that high pre-treatment levels of serum endostatin are associated with high serum VEGF levels.

Conclusion:

- Both serum VEGF and endostatin levels are significantly increased in cases of leukemia and lymphoma at diagnosis.
- Serum VEGF levels are significantly decreased after complete remission than their levels at diagnosis.
- There is no significant difference between serum endostatin levels at diagnosis and their levels after complete remission.
- No role for age or types of both leukemia and lymphoma in VEGF and endostatin expression.
- VEGF and endostatin expression has a prognostic role for follow up of cases with leukemia and lymphoma.

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