RESEARCH PAPER

Serum Vascular Endothelial Growth Factor-A Levels During Induction Therapy in Children with Acute Lymphoblastic Leukemia

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Objective: To evaluate serum vascular endothelial growth factor (VEGF) levels in children with acute lymphoblastic leukemia (ALL) during the induction phase of chemotherapy.

Design: Prospective sudy.

Setting: Hospital-based study over 18 months.

Patients: 30 children with ALL and 17 healthy age- and sex-matched controls.

Intervention: Serum concentration of VEGF-A–165 isoform (s-VEGF) was measured by enzyme-linked immunoabsorbant assay at diagnosis and at the end of induction chemotherapy.

Main outcome measures: s-VEGF was compared with markers of tumor burden. Kinetics of s-VEGF was assessed in response to induction chemotherapy.

Results: Median VEGF was significantly lower in untreated patients than in controls (17.0 *vs.* 42.6 pg/mL, *P*=0.004). s-VEGF levels were fairly correlated with WBC count (r=-0.56, *P*=0.004) and LDH (r=-0.52, *P*=0.02) at diagnosis. All patients but one were in morphologic remission at the end of induction. Median s-VEGF concentration on day 29/33 was significantly higher than on day 1 (44.2 pg/mL, *P*=0.009).

Conclusion: Untreated children with ALL have significantly lower s-VEGF concentration than controls. At the end of the induction therapy, s-VEGF increased to levels similar to controls. The role of ligand-receptor interaction between VEGF and VEGF receptors on leukemia cells needs to be further delineated.

Key words: Angiogenesis, hematological malignancy, VEGF.

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Vascular Endothelial Growth Factor (VEGF) is an essential regulator of physiologic and pathologic angiogenesis. VEGF over-expression is associated with tumor growth, invasion and metastasis in malignancies, especially solid tumors [1]. VEGF is also thought to play some role in etiopathogenesis of leukemia and multiple myeloma, as dysregulation of VEGF expression and signaling pathways are seen in these hematologic malignancies [2]. Direct and indirect inhibition of VEGF and its receptors may provide a potent novel targeted therapy to overcome resistance to chemotherapy [3].

Data on VEGF in acute lymphoblastic leukemia (ALL) patients are limited and studies show conflicting results. $VEGF_{165}$ is the principal isoform of VEGF-A, the prototype member of the VEGF family. We prospectively evaluated the levels of serum $VEGF_{165}$ (s-VEGF) in children with ALL during the induction phase of chemotherapy, in order to assess whether s-VEGF correlates with risk stratification and with treatment response.

METHODS

This prospective study was performed on newly diagnosed children 1-18 years of age with ALL in the Pediatric Hematology and Oncology Unit of Sir Ganga Ram Hospital between September 2008 and February 2010. A sex-and age-matched control population was taken. The diagnosis of ALL was based on bone marrow morphology and flowcytometry. Demographic data of cases and controls were recorded. Written informed consent was taken from parents/guardians of cases and controls. Ethical committee clearance was obtained from the institution before starting the study.

High-risk ALL was defined as white blood cell (WBC) >1,00,000/mm^3, translocation t(9;22) or Bcr-Abl recombination, t(4;11) or MLL gene rearrangement, prednisolone poor response (peripheral blasts >1000/mm^3 on day 8 or absence of morphological remission at the end of induction (bone marrow blasts >5%). Patients with none of the above were classified as standard risk group. UKALL-XI protocol (29 day-induction) was used for standard risk patients and BFM-95 protocol (33 day-induction) for high-risk patients. Complete remission was defined by the presence of <5% blasts in the bone marrow and cerebrospinal fluid clear of malignant cells at the end

of induction therapy.

Blood samples were obtained on Day 1 and Day 29/33 in ALL cases and once in controls. Serum was separated and stored at -70ºC to avoid loss of bioactive human VEGF. The Assay Designs Human VEGF Enzyme Immunometric Assay kit was used to assess serum concentration of $VEGF_{165}$.

Mann-Whitney U-test or Kruskal-Wallis test were applied to compare initial s-VEGF level in patients and controls, and between various patient groups. Correlation of s-VEGF level with other continuous variables at diagnosis was done by Spearman's correlation coefficient. Comparison of s-VEGF on day 1 and on day 29/33 was done by Wilcoxon signed-rank test.

RESULTS

The study group comprised of 30 children with ALL (***Table* I** and ***Web Table* I**). The control group had 17 healthy children (9 males and 8 females) with a median age of 5 years (range 2–12 years). The median s-VEGF value was significantly lower in patients than in controls (***Table* II**). At the time of ALL diagnosis, median s-VEGF did not differ significantly between sex, age groups, immunophenotypes and ploidy on bone marrow cytogenetics.

There was a fair inverse correlation of s-VEGF levels with absolute WBC count at diagnosis (Spearman's correlation coefficient -0.56, *P*=0.004) and LDH (r=-0.52, *P*=0.02). There was no correlation between s-VEGF and hemoglobin or platelet count. Patients with high-risk and intermediate risk ALL showed lower s-VEGF levels than those with standard-risk (8.6 pg/mL [interquartile range-IQR 0.2-48.2] *vs*. 24.8 [IQR 10.8-53.5]), but the difference was not statistically significant (*P*=0.2).

All patients but one were in morphologic remission at the end of induction. Median s-VEGF at the end of induction therapy was significantly higher than at diagnosis (***Web Table* I**) and was not significantly different from s-VEGF in controls (*P*=0.91). The patient that did not attain remission was a 5-year old boy with pre-B phenotype, low WBC, and normal cytogenetics. His s-VEGF on day 1 and day 29 were 10.7 and 59 pg/mL, respectively.

DISCUSSION

Our results show an inverse correlation between s-VEGF concentration at the time of diagnosis and disease burden, as suggested by increased WBC counts and LDH. We also found lower levels of s-VEGF at the time of diagnosis of ALL as compared to the end of induction therapy. With the attainment of remission, s-VEGF rose to levels almost similar to those of healthy controls. Our results corroborate the study by Yetgin, *et al*. [4] wherein the median level of s-VEGF at the time of diagnosis was significantly lower than those of the control group and of the patients in remission, with no relation between Hb, WBC, absolute blast percentage and s-VEGF. Likewise, Aref, *et al.* [5] found significantly lower s-VEGF levels in pre-B ALL newly diagnosed patients at diagnosis than in controls, levels which increased to near controls in remission. Considering the fact that VEGF is expressed in normal hematopoietic cells, we hypothesize that in children suffering from ALL, the proportion of hematopoietic cells is decreased in comparison with tumor cells and consequently, the level of VEGF may be lower. However, during remission, the renewal of normal hematopoiesis may explain the rise of s-VEGF near normal levels, as evidenced in these reports and in our study [4,5].

TABLE I CHARACTERISTICS OF 30 ALL PATIENTS AT DIAGNOSIS

Characteristic	*Value*
Age, median (range)	6 years (1–14)
Male sex	20 (66.7)
ALL immunophenotyping	
Pre-B CALLA positive ALL	26 (86.7)
Pre-B Ph1 positive ALL	1 (3.3)
T-cell ALL	3 (10.0)
WBC	2,600 (700 – 314,000)
<20,000/mm^3	21 (70.0)
20,000 - 100,000/mm^3	7 (2.3)
>100,000/mm^3	2 (6.7)
LDH (*n*=26) > 2 ULN	15 (53.8)
CNS disease	1 (3.3)
Cytogenetics (n=26)	
Hyperdiploidy >51	7 (26.9)
46,XX / 46,XY	15 (57.7)
Unsuccessful culture	4 (15.4)
TEL-AML1 (n=21)	
FISH Positive	3 (14.3)
Bcr-Abl fusion* (*n*=20)	1 (5.0)
Protocol	
UKALL-XI	27 (90.0)
BFM-95	3 (10.0)
Risk group	
Standard risk	27 (90.0)
High risk	3 (10.0)

*Notes. *LDH normal value 91-180 IU/ml. ULN: upper limit of normal; *by FISH (qPCR).*

TABLE II SERUM VEGF IN ALL CASES AND CONTROLS

VEGF (pg/mL)	*Cases at diagnosis (N=30)*	*Controls (N=17)*	*Cases at end of induction (N=30)*	*P-value*
Median (IQR)	17.0 (3.0 – 47.2)	42.6 (32.0 – 74.7)		0.004
Median (IQR)	17.0 (3.0 – 47.2)		44.2 (16.7 – 102.3)	0.009

Another hypothesis that may explain our findings relates to the interaction between VEGF receptors (VEGFR) and VEGF [6]. In CLL and non-Hodgkin lymphomas, receptors present in soluble form in the plasma bind the ligand before it can bind to the actual cell-bound receptor and thus decrease the activity of angiogenic factors such as VEGF [7,8]. Both VEGF and VEGFR-1 are expressed on leukemia cells, including ALL cells [9]. Recent data from children enrolled in anti-angiogenic Children's Oncology Group (COG) clinical trials showed a significant increase of plasma VEGF and decrease of soluble VEGFR-2 after treatment with tyrosine kinase inhibitors targeting VEGF receptors [10]. These results lead us to hypothesize that low s-VEGF levels in our untreated children with ALL may reflect a higher VEGFR expression on leukemia cells that would bind s-VEGF, thus decreasing unbound s-VEGF proportionately to tumor burden via ligand-receptor interaction. Further studies on VEGFR-1 and VEGFR-2 expression are required to confirm such hypothesis.

A few clinical studies on VEGF in pediatric hematological malignancies report results conflicting with ours. Yang, *et al*. [11] found significantly higher plasma VEGF concentrations in children with ALL before treatment than in normal controls. A COG study showed that standard-risk ALL pediatric patients [12] with low VEGF-A levels at diagnosis and at the end of induction had significantly superior event-free survival (EFS). Furthermore, patients who had an increase in VEGF-A during induction had poorer EFS [12]. A similar association of high s-VEGF level and poor overall and relapse-free survival were described in childhood ALL[1,] [11, 13] and AML patients [14].

The possible explanation for the above observations and ours is a clearance of abnormal cell population from the bone marrow as the treatment progresses and reestablishment of normal hematopoiesis. Hematopoiesis is greatly disturbed in the bone marrow microenvironment when there is an excessive malignant proliferation of blasts and this probably hinders the normal marrow angiogenesis, leading to low levels of angiogenic peptides. With the action of multiagent chemotherapy, this abnormal cell population gives way to normal angiogenesis and hematopoietic activities in the bone marrow.

Our study has certain limitations. The most important one is the small number of patients included in the study, which may be the cause for non-statistically significant differences after risk stratification. Furthermore, the cohort consisted mostly of patients who attained remission at the end of induction therapy, so the possible relationship between resistant disease and VEGF levels could not be attained.

Further investigations on the role of angiogenesis and angiogenic factors in childhood ALL will provide additional information in understanding the complex interaction between angiogenesis and the biology of leukemic cells. This understanding can eventually lay the pathways for clinically helpful targeted molecules to further improve the outcome of pediatric leukemias.

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What is Already Known?

- Vascular endothelium growth factor (VEGF) plays an important role in tumor angiogenesis in solid tumors but its role in leukemia is not well known.

What This Study Adds?

- Serum VEGF levels are low at diagnosis and increase to normal levels after end of induction chemotherapy in children with acute lymphoblastic leukemia.

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