Original Article

Correlation between Soluble Vascular Endothelial Growth Factor A and its Receptor 1 with Response to Chemotherapy in Acute Leukemia in Children

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ABSTRACT

Background and Objective: **Vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1 and R2) are major regulators of angiogenesis. This study was designed to assess serum levels of VEGF and VEGF-R1 and their prognostic significance in newly diagnosed childhood acute leukemia.**

Materials and Methods: **For this purpose, VEGF and VEGF-R1 were determined using enzyme linked immuno-sorbant assay (ELISA) in samples obtained before treatment. Demographic data were recorded. Bone marrow blast percentage was counted on diagnosis and 2 weeks after induction therapy. A p value less than 0.05 considered significant.**

Results: **Fifty-three children (22 boys and 31 girls) with newly diagnosed acute leukemia were enrolled in the study. Most cases (56.6%) were pre B cell ALL. Mean value of VEFG-A in good responders was 55.13 ± 24.96 pg/ml and in poor responders it was 94.46 ± 15.75 ($p<0.0001$). Mean valve of VEGF-R1 in good and poor responders was 0.132 ± 0.0653 and 0.1665 ± 0.0857 pg/ml respectively ($p>0.05$). Using ROC curve, we found out a cut-off point of 76 pg/ml to discriminate poor response to chemotherapy.**

Conclusion: **Soluble VEGF-A is an independent factor for response to therapy in childhood leukemia and leukemic patients with sVEGF-A level over 76 pg/ml will have poor response to treatment.**

Key words:Vascular Endothelial Growth Factor Receptor-1, Leukemia, Child, Vascular Endothelial Growth Factor A

Introduction

Vascular endothelial growth factor (VEGF) is a family of peptides that includes VEGF-A (referred as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. VEGF is a potent inducer of blood vessel formation in early development (vasculogenesis) and has a central role

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in the growth of new blood vessels (angiogenesis) in adults. It promotes angiogenesis in tumors, chronic inflammation and wound healing. Members of VEGF family signal through three tyrosine kinase receptors: VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 is located in endothelial cells and is the main receptor for the vaculogenic and angiogenic effects of VEGF (1).

Tumors stimulate the growth of host blood vessels, which is essential for supplying nutrients to the tumor. Angiogenesis is a requisite not only for continued tumor growth but also for metastasis (2). Over-expression of VEGF is associated with increased angiogenesis, growth and metastasis in solid tumors. VEGF is also secreted by a variety of tumor cell lines including hematopoietic cells, but the significance of VEGF in leukemia has received only limited attention (3). Recent studies have suggested that angiogenesis may also be involved in the pathogenesis of certain hemic malignancies. One study concerning childhood acute lymphoblastic leukemia (ALL) revealed an increased microvessel in patient's bone marrow compared with normal tissue. Increased bone marrow microvessel density was also observed in a series of patients diagnosed as multiple myeloma and hairy cell leukemia (4). The prognostic significance of cellular VEGF expression in chronic phase of chronic myeloid leukemia has been shown by Verstovsed et al (5). In addition, other studies have shown that in acute myeloid leukemia there is an increased expression of VEGF-C and its receptor, VEGF-R3 in the bone marrow of the patients that display no prognostic significance (6). Hou et al showed that expression of Ang-2, Ang-1, and also VEGF-A were elevated in BM leukemia cells of patients with newly diagnosed AML compared with normal controls (7).

Several therapeutic approaches targeting the VEGF/VEGF-receptor system have recently been developed. Thus, it is important to understand the significance of VEGF in acute leukemia to better define the potential role of these approaches in leukemia therapy (2;8). Kini et al showed that all-trans retinoic acid (ATRA) therapy inhibits VEGF production and suppresses angiogenesis in acute promyelocytic leukemia (9).

Although the majority of studies have been conducted on microvascular density in bone marrow, but there are soluble factors in serum that may have significance and moreover leukemia is the most common malignant neoplasm of infancy and childhood, so the aim of this study was to examine the possible correlation between serum soluble VEGF-A and VEGF-R1 and response to therapy in newly diagnosed acute leukemia in children.

Materials and Methods

A prospective cross-sectional study was designed among newly diagnosed children with acute leukemia between 2006 and 2007 in Ali-Asghar children hospital. The diagnosis was based on the presence of blasts in at least 20% of the marrow nucleated cells. All patients who were suspicious to leukemia, because of the abnormalities in complete blood count, underwent bone marrow aspiration with confirmatory flow cytometric study at the time of initial presentation. The patients were enrolled in the study by non-probability and sequential sampling before receiving any therapy. The consents were taken from parents or patients.

Data of clinical findings, complete blood count and bone marrow aspiration at the time of initial presentation were recorded. Before starting any therapy 3 ml of anti-coagulated blood was drawn from each patient, the serums were separated and freezed at -70 ºC until being tests.

All patients were treated with standard chemotherapies. Most patients, except B-cell ALL and infants younger than 1 year at the time of diagnosis, received modified BFM (British, French, Munich) 76/79 protocol. In the first 5 weeks of this protocol, named as induction phase or phase one, the patients received drugs like vincristin, daunorubicin (daunomycin), L-asparginase and prednisone and response to chemotherapy and presence of remission were evaluated by a second bone marrow aspiration for determining blast count reduction to less than 5% (10). In addition, in our patients, on day 15th of initiation of induction chemotherapy, the second sample of bone marrow aspiration was drawn and blast cells were counted in at least 500 marrow nucleated cells at high power magnification(X400) using light microscopy. VEGF-A and VEGF-R1 were assessed by BOILISA (biotin-strepavidin HRP) assay (11), with Bender-Medsystem kits (Denmark) and the change of color was read with photometer at 450 nm. All samples were run in duplicate.

All statistical analyses were performed using SP-SS (version 11) and student t test and for paired of categorical variables by the fisher exact and chi-square tests. P value less than 0.05 was considered statistically significant.

Results

Fifty-three children (22 boys and 31 girls) with newly diagnosed ALL with a median age of 6.4 ± 3.72 years were enrolled in the study. Table 1 lists the clinical characteristics of the patients.

Table 1: Clinical characteristics of the studied population

Clinical characteristics	Number
Sex	53
Female	31
Male	22
Median age (yr)	6.4 ± 3.72
Immunological subtype (%)	
Pre B-cell ALL	33 (62.3%)
T-cell ALL	9 (17%)
AML	7 (13.2%)
B-ALL	4 (7.5%)
Splenomegaly	12
WBC count (/μL)	12700
RBC count (x10^3/μL)	3640
Hb (g/dl)	8.4 (± 2.42)

The mean values for VEGF-A and VEGF-R1 were 75.2 ± 28.6 pg/ml and 0.142 ± 0.083 pg/ml respectively. The average of bone marrow blast count at the time of diagnosis was 63.35% ± 28.61 and this figure was 12.37% ± 13.42 two weeks after treatment (paired t test, $p<0.0001$). Meanwhile, 26 (49.1%) out of patients showed good response to therapy and had marrow blast count less than 5%.

Mean value of VEFG-A in good responders was 55.13 ± 24.96 pg/ml, while in poor responders it was 94.46 ± 15.75 (t test, $p<0.0001$). Mean valve of VEGF-R1 in good and poor responders were 0.132 ± 0.0653 and 0.1665 ± 0.0857 pg/ml respectively ($p>0.05$).

There was no correlation between age, type of leukemia, presence of splenomegaly with mean values of VEGF-A and its receptor. There was also no correlation between sVEGF-A and sVEGF-R1 level. The mean of sVEGF-A showed direct strong correlation with blast count two weeks after therapy ($r = 0.85$, $p<0.001$) but reverse weak correlation with mean of RBC count at the time of diagnosis ($r = -0.28$, $p=0.03$). We did not find any correlation between sVEGF-A and sVEGF-R with mean platelet, WBC or blast count at the time of diagnosis. Using ROC curve, we found cut off point 76 pg/ml (area under curve = 0.824) with a sensitivity of 99% and a specificity of 81% to discriminate poor response to chemotherapy.

Discussion

Our present study demonstrated that high level of serum sVEGF-A has reverse correlation with response to therapy; whereas there is no correlation between serum sVEGF-R1 and response.

Aguayo et al report was one of the first studies about this factor. They found out that there was a relationship between increasing VEGF levels and shorter survival, as well as disease-free survival, both from start of treatment and from complete response data. In contrast, there was no relationship between VEGF level and WBC or blast count or between VEGF level and such established prognostic factors. Their result suggested that at least in AML patients with higher WBC and blast counts, cellular VEGF level is an independent predictor of outcome (12). Koomagi et al found that VEGF levels were significantly higher in recurrent ALL compared with the newly diagnosed ALL, while median relapse-free survival in low VEGF group was more than high VEGF group (3).

We also found a direct correlation between soluble VEGF-A and response to therapy with a cut off point of 76 pg/ml, a sensitivity of 99% and a specificity of 81%. There was no correlation between sVEGF-A and sVEGF-R1 in childhood ALL, so these two factors works independently. Moreover, there was no correlation between sVEGF-R1 and response to therapy. Hu et al also reported that increased plasma levels of soluble VEGFR2 correlated with a lower rate of complete remission in patients with MDS but not in patients with AML. VEGFR1 but not VEGFR2 was independent prognostic factors in both patients with AML and patients with MDS (13). Ferrajoli et al found cellular VEGF-R2 levels serve as a prognostic factor in chronic lymphocytic leukemia (14), so further studies should investigate relationship between childhood leukemia and VEGF R2 that was impossible for us in this study. There was also no correlation between VEGF-A level and other prognostic factors like age and ALL subtype. Reverse correlation between VEGF-A and RBC count could be because of suppressive effect of blasts on hematopoiesis. In Avramis et al study, VEGF-A serum levels <40 and >100 pg/ml have been

associated with good and poor prognoses respectively (15). According to results of this study, measuring this factor in serums of leukemic patients, which can be assayed in a very simple way, may predict the response to chemotherapy and therefore prognoses of the patients. In the patients with high levels of VEGF-A, the clinicians can change standard chemotherapy using more numerous and vigorous chemotherapeutic agents.

Conclusion

Soluble VEGF-A is an independent factor indicative of response to therapy in childhood leukemia and a serum level above 76 pg/ml provide poor response. There is no correlation between serum levels of sVEGF-A and sVEGF-R1 as well as VEGF-R1 and response to therapy in childhood leukemia.

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