Paediatrica Indonesiana

p-ISSN 0030-9311; e-ISSN 2338-476X; Vol.59, No.4(2019). p. 217-21; doi: http://dx.doi.org/10.14238/pi59.4.2019.217-21

Original Article

Vascular endothelial growth factor (VEGF) expression in induction phase chemotherapy of acute lymphoblastic leukemia

Dasril Daud, Merlyn Meta Astari, Nadirah Rasyid Ridha

Abstract

Background Leukemia is a hematolymphoid malignancy originating from bone marrow. The progression of hematolymphoid malignancies depends on new formation of vasculature, called angiogenesis. Angiogenesis is regulated by vascular endothelial growth factor (VEGF), which is secreted by paracrine and autocrine signaling mechanisms.

Objective To evaluate VEGF expression in induction phase chemotherapy of acute lymphoblastic leukemia (ALL) patients.

Methods This prospective, cohort study was conducted in ALL patients admitted to Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, from October 2016 to October 2017. Subjects' VEGF levels were measured at diagnosis and at the end of induction chemotherapy.

Results VEGF levels were analyzed in 59 patients, 29 of whom were diagnosed with standard risk ALL and 30 patients with high risk ALL. VEGF levels were significantly decreased after induction phase chemotherapy in standard risk ALL and in high risk ALL subjects. There was no significant difference in VEGF levels before induction phase chemotherapy between the standard and high risk groups (P=0.405). There was also no significant difference in VEGF levels after induction phase chemotherapy between the two risk groups (P=0.094).

Conclusion The VEGF level is significantly lower after ALL induction phase chemotherapy in both the standard risk and high risk ALL groups. However, there are no significant differences in VEGF levels between the standard and high risk groups before as well as after induction phase chemotherapy. **[Paediatr Indones. 2019;59:217-21; doi: http://dx.doi.org/10.14238/pi59.4.2019.217-21]**.

Keywords: *angiogenesis; acute lymphoblastic leukemia; VEGF*

Acute leukemia is defined as a blood cell malignancy originating from the bone marrow, and characterized by proliferation of white blood cells with abnormal cell manifestations in peripheral blood and extramedullary sites. Leukocytes in the bone marrow proliferate irregularly and uncontrollably. In addition, leukocyte function becomes abnormal. Because of this process, other functions of normal blood cells are also disrupted, causing leukemia symptoms.[1,2] Acute leukemia in children comprises approximately 30-40% of malignancies in children, and can occur at all ages. The highest incidence generally occurs at the age of 2-5 years, with an average incidence of 4-4.5 cases/year/100,000 children under the age of 15. Although the exact cause of acute lymphoblastic leukemia is still unknown, several factors are involved, including

From the Department of Child Health, Universitas Hasanuddin Medical School/Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia.

Corresponding author: Dasril Daud. Department of Child Health, Universitas Hasanuddin Medical School/Dr. Wahidin Sudirohusodo Hospital, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea Makassar, 90245, South Sulawesi, Indonesia. Tel. +62411-584461; Email: unhaspediatrics.hi@gmail.com/drdasril@gmail.com.

Submitted March 27, 2019. Accepted August 14, 2019.

endogenous and exogenous exposure (to ionizing radiation, pesticides certain solvents or viruses), genetic involvement, and risk factors.[1,3]

Angiogenesis is the process of capillary formation of blood vessels which is important in wound healing, development, reproduction, and hematological malignancy. The VEGF over-expression is associated with tumor growth, invasion, and metastasis in malignancy, especially with regards to solid tumors. The growth and survival of cancer cells are influenced by supply of oxygen, nutrients, and VEGF by endothelial cells in angiogenesis. Previous studies observed that tumor growth can be suppressed by inhibition receptor of VEGF, therefore no angiogenesis signal is produced.[3] Vascular endothelial growth factor (VEGF) is a heparin-binding, homodimeric glycoprotein or ligand with five components: VEGF-A (or VEGF) with several isoforms, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF). The VEGF shows target cell specificity to endothelial cells. Some cell types, including fibroblasts, endothelial cells, and keratinocytes produce VEGF in small amounts.[4,5]

The progression of hematolymphoid malignancies depends on the induction of new blood vessel formation, such as in acute leukemia, myelodysplasia syndrome, multiple myeloma, and lymphoma.[6] The VEGF is the most important proangiogenic agent. It activates receptors in vascular endothelial cells and increases blood vessel regeneration.[7] The angiogenesis process begins with the release and formation of angiogenic growth factors that diffuse around the damaged tissue. This angiogenic growth factor then binds to specific endothelial cell receptors in the nearby blood vessels and signals growth from the cellular surface to be transmitted to the nucleus. Furthermore, endothelial cells produce new molecules including various enzymes that dissolve proteins and form small holes in the basement membrane to proliferate.[3]

The survival of cancer cells depends heavily on nutrient and oxygen intake. Such cancer cells develop angiogenesis through VEGF production. The VEGF can be produced by the tumor cells themselves, to act through paracrine or autocrine signalling mechanisms. In addition, endothelial cells produce normal VEGF and have receptors that bind to VEGF (VEGF-R2). The main mediators of tumor angiogenesis are specifically the VEGF 121 and 165 isoforms. These isoform signals pass through the VEGF 2 receptor (VEGFR-2), the main receptor mediating the process of angiogenesis. Many cancer cells express VEGF, and increased tumor expression is often associated with a poor prognosis.[8] The VEGF signals mainly through VEGF receptor 2 (VEGFR-2), which is expressed at elevated levels by endothelial cells engaged in angiogenesis and by circulating bone marrow-derived endothelial progenitor cells. The detailed structure of VEGFR-2 in endothelial cells is unknown, but receptors are usually described as functioning to bind signal molecules and located on cellular surfaces. Chemotherapy is beneficial for treatment of hematological malignancies, as it destroys malignant cells, decreasing production of VEGF.[9] Therefore, it is important to know the VEGF levels before and after induction phase chemotherapy. Kalra *et al*.[6] showed that VEGF levels were higher in relapse than at diagnosis, but such studies have been limited. Since VEGF plays a role in angiogenesis to supply cancer cell requirements, leukemia patients are at risk of cachexia. Moreover, decreased VEGF levels produced by cancer cells after chemotherapy, are an indication of good response to treatment. However, VEGF levels that do not change or remain high indicate that leukemia cells are still proliferating and/or the patient is at risk of relapse.[9]

This study aimed to evaluate VEGF expression in induction phase chemotherapy of acute lymphoblastic leukemia (ALL) patients.

Methods

This prospective cohort study was conducted in DR Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, during October 2016-October 2017. VEGF levels were compared before and after induction phase chemotherapy in patients with acute lymphoblastic leukemia (ALL). Subjects' parents provided informed consent for study inclusion. The Ethics Commission of Hasanuddin University and Dr. Wahidin Sudirohusodo Hospital approved the study.

Subjects were patients with an ALL diagnosis aged 1 month to 18 years. Twenty-one patients who did not complete chemotherapy were excluded (15 died, 2 refused chemotherapy, and 4 dropped out of chemotherapy).

Patients aged 1 month - 18 years were diagnosed based on clinical symptoms and the results of complete blood examination and bone marrow puncture (BMP). Data recorded were age, sex, weight, body length/height, nutritional status, vital signs (temperature, pulse, blood pressure, breathing, and consciousness), and routine blood tests. There were classified according to the risk into two groups: a standard-risk group (SR) and a high-risk group (HR). Inclusion criterias for high risk ALL: age < 1 year or > 10 years, leucocyte ≥ 50,000 cells/mm^3 of blood, > 5 leukemic cells in CSF, testicular involvement, mediastinal mass, and leukemia cells with chromosome changes. The standard risk ALL inclusion criterias : age 1-10 years, leucocyte < 50,000 cells/mm^3, < 5 leukemic cells in CSF, no testicular involvement, no mediastinal mass, and no chromosome changes in leukemia cells. Blood specimens were taken at Day 1 before induction phase and at Day 42/44 after induction phase of chemotherapy for VEGF measurements. Serum was separated and stored at -80° C to avoid loss of bioactive human VEGF. The Assay Designs Human VEGF Enzyme Immunometric /assay kit was used to assess serum consentration of VEGF.

Data were grouped based on the purpose and type into the appropriate ALL group, as well as before and after chemotherapy. Univariate analysis, bivariate analysis, and correlation test were used for statistical analyses.

Results

Of 80 patients aged 1 month to 18 years diagnosed with ALL, 59 patients met the inclusion criteria, including 29 patients with standard risk (SR) and 30 patients with high risk (HR). The SR group consisted of 16/29 males while the HR group consisted of 20/30 males. The ALL SR subjects were aged 1-10 years, while the HR subjects were mostly aged 1-10 years (17/30), followed by ≥ 10 years (12/30), and <1 year (1/30). Nutritional status of the SR group consisted of 14/29 well-nourished subjects, 8/29 undernourished subjects, and 7/29 malnourished subjects; the HR group had 17/30 well-nourished subjects, 4/30 undernourished subjects, 7/30 malnourished subjects, and 2/30 overweight subjects (**Table 1**).

Median VEGF levels in the standard risk ALL group were 1,686 (range 217-14,879) ng/L before chemotherapy and 971 (range 450-2,880) ng/L after chemotherapy. Wilcoxon test revealed a significant difference in VEGF levels before and after chemotherapy (P=0.000) (**Table 2**).

Median VEGF levels in the high risk ALL group level were 2,866.5 (range 92-16,452) ng/L before chemotherapy and 1,238 (range 702-2,698) ng/L after chemotherapy. Wilcoxon test revealed a significant difference in VEGF levels before and after chemotherapy in the HR group (P=0.001) (**Table 3**).

Wilcoxon test revealed no significant difference in VEGF levels before chemotherapy between the SR and HR groups (P=0.405). In addition, Wilcoxon test

Table 1. Characteristics of subjects

Characteristics	Acute lymphoblastic leukemia	
	Standard risk (n=29)	High risk (n=30)
Sex		
Male	16	20
Female	13	10
Nutritional status		
Well-nourished	14	17
Undernourished	8	4
Malnourished	7	7
Overweight	0	2
Age		
< 1 year	0	1
1- 10 years	29	17
>10 years	0	12

Table 2. Comparison of VEGF levels before and after chemotherapy in SR patients

VEGF level, ng/L	Standard risk		P value
	Before chemotherapy	After chemotherapy	
Mean (SD)	3,206.8 (3,901.3)	1,154.3 (477.2)	0.000
Median (range)	1,686 (217-14,879)	971 (450-2,880)	

Wilcoxon test (P<0.05)

Table 3. Comparison of VEGF levels before and after chemotherapy in HR patients

VEGF level, ng/L	High risk		P value
	Before chemotherapy	After chemotherapy	
Mean (SD)	3,377.3 (3365.6)	1,398 (555.1)	0.001
Median (range)	2,886.5 (92-16,452)	1,238 (702-2,698)	

Wilcoxon test (P<0.05)

Table 4. Comparison of VEGF levels between the SR and HR groups before and after chemotherapy

VEGF level, ng/L	Before chemotherapy		P value	After chemotherapy		P value
	Standard risk	High risk		Standard risk	High risk	
Mean (SD)	3,206.8 (3,901.3)	3,377.3 (3,365.6)	0.405	1,154.1 (477.2)	1,398 (555.1)	0.094
Median (range)	1,686 (217-14,879)	2,886.5 (92-16,452)		971 (450-2,880)	1,238 (702-2,698)	

Wilcoxon test (P>0.05)

revealed no significant difference in VEGF levels after chemotherapy between the two groups (P=0.094) (**Table 4**).

Discussion

The VEGF levels were significantly reduced in SR and HR ALL patients after induction phase chemotherapy. This finding may suggest that chemotherapy has an anti-angiogenic effect in these patients.

We should note that the authors did not look at vascularization before and after. Angiogenesis is the process of forming new capillaries from blood vessels and is an important process in development, wound healing, and reproduction. It is also an important factor in hematological malignancies, including acute and chronic leukemia, multiple myeloma, and myelodysplasia syndrome. Excessive expression of VEGF is associated with tumor growth, invasion, and metastasis in malignancies, especially with regards to solid tumors.[2] The growth and survival of cancer cells are influenced by supply of oxygen, nutrients, and VEGF by endothelial cells in angiogenesis.[3] The key, pro-angiogenic mediator of the angiogenesis process is VEGF. The VEGF is a glycoprotein that stimulates angiogenesis and capillary permeability by binding to tyrosine kinase receptors 1 and 2. Many leukemias are associated with angiogenesis, since HL60 cells from acute myeloid leukemia are used for gene clones.[3]

Acute lymphoblastic leukemia management is divided into standard risk and high risk groups. Current conventional chemotherapy is used as an antiangiogenic therapy for leukemia cells. Antiangiogenic therapy used as monotherapy has proven to have no effect or benefit in the treatment of hematological malignancies so far. However, some antiangiogenic therapies combined with conventional chemotherapy have been shown to be effective in suppressing the angiogenesis process.[8]

Of 59 subjects in our study, 29 subjects were categorized to have standard risk ALL, with a male: female ratio of 1.3: 1, and 30 subjects were categorized to have high risk ALL with a male: female ratio of 2: 1. Kamima *et al.*[11] in Cipto Mangunkusumo Hospital reported a similar male: female ratio of 1.3: 1 of ALL patients.[10] Also, Silawati in Wahidin Sudirohusodo Hospital reported a ratio of 1.5: 1.

Malnutrition has an impact on multifactorial prognosis. Variance of body composition of lean body mass and adipose tissue will affect the pharmacokinetic and pharmacodnamic of many drugs we use. Previous studies showed that nutritional status should be considered by treating pediatric oncologist.[12] In our study most subjects with standard risk ALL were well-nourished, followed by undernourished, and malnourished. High risk ALL subjects were also mostly well-nourished, while the rest were undernourished, malnourished, and overweight. However, in our study we did not analyze correlation between nutritional status and prognosis.

Angiogenesis has proven to be an important process in hematological malignancies. Several studies have reported an association between leukemia and angiogenesis, since HL60 cells, the acute myeloid leukemia cell line, are used as clones of the VEGF gene. Increased bone marrow vascularization has been found in acute and chronic leukemia in both adults and children.[13]

Median VEGF level in standard risk protocol patients was significantly decreased after chemotherapy compared to before chemotherapy. Leblebisatan *et al.*[14] found that VEGF was higher in leukemia subjects than in healthy subjects. In addition, VEGF levels were not significantly different between AML and ALL patients, but VEGF levels at remission were lower than at the time of initial diagnosis.[12] Also, Giles15 noted that VEGF levels in 417 patients with acute myeloid

leukemia (AML) and myelodysplastic syndromes (MDS) were higher compared to controls.

Median VEGF level in high risk protocol patients was significantly decreased after chemotherapy compared to before chemotherapy. The decreased VEGF levels after chemotherapy may suggest that chemotherapy successfully suppresses angiogenesis in leukemia. Conventional chemotherapy was used as an antiangiogenic therapy for leukemia cells. However, some antiangiogenic therapies combined with conventional chemotherapy have been effective in suppressing the angiogenesis process.[9]

The weakness of this study was that we only measured VEGF level without considering other anti-angiogenesis effects. The strength of this study was its prospective cohort design which is useful for determining the course of the disease or the effects studied in explaining the risk dynamics and outcome effects. Our findings also provide data on VEGF levels in both standard risk and high risk ALL patients. Thus, reference VEGF levels may be useful in future studies on possible associations between leukemia, angiogenesis, and chemotherapy. Further studies on association between VEGF and remission/relapse are required. Multi-center or meta-analysis studies in other hospitals in Indonesia need to be done in order to validate our findings.

In conclusion, VEGF levels significantly decrease after chemotherapy, both in standard risk and high risk ALL patients. There is no significant difference in VEGF levels in standard risk and high risk ALL patients before chemotherapy as well as after chemotherapy.

Conflict of Interest

None declared.

Funding Acknowledgment

The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Inaba H, Greaves M, Mulighan CG. Acute lymphoblastic leukemia. Lancet. 2013;381:1943-55.
2. Terwillinger T, Abdul-Hay M. Acute lymhoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017;7:e577.
3. Schneider P, Dubus I, Gouel F, Legrand E, Vannier JP, Vasse M. What role for angiogenesis in childhood acute lymphoblastic leukaemia? Adv Hematol. 2011;2011:274628.
4. Kresno SB. Angiogenesis. In : Ilmu Dasar Onkologi. 3th ed. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia;. 2012. p 344-68.
5. Sukhramani PS, Suthar MP. VEGF inhibitors for cancer therapy. Int J Pharmaceut Sci Drug Res. 2010;1:1-11.
6. Kalra, M, Dinand V, Choudary S, Sachdeva A, Yadav SP. Serum vascular endothelial growth factor-A levels during induction therapy in children with acute lymphoblastic leukemia. Indian Pediatr. 2013;50:659-62.
7. Peach CJ, Mignone VW, Arude MA, Alcotia DC, Hill SJ, et al. Molecular Pharmacology of VEGF-A Isoform : Binding and Signaling of VEGFR2. Int. J.Mol Sci. 2018;19:1264.
8. Kerbel RS. Tumor angiogenesis. New Engl J Med. 2008; 358:2039-49.
9. Ribatti D. Angiogenesis as a treatment target in leukemia. Int J Hematol Oncol. 2013;2:229-42.
10. Kamima K, Gatot D, Hadinegoro S. Profil antioksidan dan oksidan pada anak dengan leukemia limfoblastik akut pada kemoterapi fase induksi (studi pendahuluan). Sari Pediatri. 2009;11:282-8.
11. Silawati T. Ridha NR, Daud D. Correlation of sex and remission of acute lymphoblastic leukemia-l1 (all-l1) in children. International Journal of Clinical and Experimental Medical Sciences. 2015;1: 11-5. DOI: 10.11648/j.ijcems.20150102.12.
12. Rogers P. Nutritional status as a prognostic indicator for pediatric malignancies. Clinical Oncolgy J. 2014;32:1293-4.
13. Mansour A, Shehata H, Ali M, Sallam M, El Khouly N, Asfour I. A novel approach to acute lymphoblastic leukemia in adults: association analysis of polymorphisms in vascular endothelial growth factor (VEGF) gene and clinical outcome. Int J Cancer Res. 2015;11:93-103.
14. Leblebisatan G, Antmen B, Sasmaz I, Kilinc Y. Vascular endothelial growth factor levels in childhood acute lymphoblastic and myeloblastic leukemia. Indian J Hematol Blood Transfus. 2012;28:24-8.
15. Giles FJ. The vascular endothelial growth factor (VEGF) signaling pathway: a therapeutic target in patients with hematologic malignancies. Oncologist. 2001;6:32-9.