Original Article

Iraqi National Journal of Medicine. July 2021, Vol. 3, Issue 1

Evaluation of VEGF-A in relation to childhood acute lymphoblastic leukemia (in Basrah, Iraq)

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ABSTRACT

Background: Cancer angiogenesis demonstrates an important role in the progression and pathogenesis of blood malignant disorders including acute lymphoblastic leukemia (ALL). Vascular endothelial growth factor (VEGF)-A is one of the most effective elements of endothelial cell growth; it promotes vascular permeability of endothelial cells and provides the new vasculature with oxygen and nutrients. Higher VEGF-A levels in childhood acute lymphoblastic leukemia (ALL) is associated with poorer patient outcomes.

Aim of the study: to assess the level of VEGF-A in plasma of children with ALL.

Subject and method: Forty children with ALL and 40 healthy children as control were enrolled in this study conducted at the Oncology Unit in Basrah Children's Hospital from Oct 2019 to March 2020. Plasma VEGF-A level was evaluated using ELISA assay.

Results: The plasma level of VEGF-A is higher in ALL children than those in the control ($p < 0.001$). Moreover, the plasma VEGF-A level in the high-risk group (HRG) is higher than that in the standard risk group (SRG).

Conclusion: The significantly higher level of plasma VEGF-A in ALL children compared to the healthy ones may demonstrate the role of VEGF-A in stimulating angiogenesis in pediatric ALL.

Keywords: Angiogenesis, vascular endothelial growth factor-A, childhood acute lymphoblastic leukemia, acute lymphoblastic risk groups.

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Disclaimer: The authors have no conflict of interest.

DOI: https://doi.org/10.37319/iqnjm.3.1.4
Received: 8 Sep 2020 Accepted: 12 Nov 2020 Published online: 15 Jan 2021

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the bone marrow malignant disorder in which the normal hematopoietic marrow cells are replaced by early lymphoid precursors (1). ALL is the most common kind of malignancy in adolescents and children accounting for 23–25% of all cancerous disorders (2).

Angiogenesis is an active step during physiological processes and human pathologies such as tumor development and metastasis (3). It includes activation of endothelial cells of a mature vessel, the movement of neighboring vascular endothelial cells, localized destruction of the adjacent basement membrane, and growth to the formation of newly produced blood vessels (4). Vascular endothelial growth factor A (VEGF-A) is considered an important growth factor among the other factors that are involved in both vasculogenesis and angiogenesis (5). The production of hypoxia-inducible factor (HIF) by cells that do not receive sufficient oxygen causes the VEGF-A release. VEGF-A plays a critical role in neovascularization induction in hematologic malignancies stimulating tumor progression and metastatic potential (6). However, the role of angiogenesis in ALL children is controversial; some researchers have shown that in children with ALL, the VEGF-A of leukemic cells promotes angiogenesis (7). As a result, this stimulates both the blood vessel formation within the bone marrow and the proliferation of the malignant cells (8).

This study aims to assess the VEGF-A plasma level in childhood ALL and compare it in a different risk group.

SUBJECT AND MATERIAL

This study is a case-control study conducted from October 2019 to March 2020. To assess the VEGF-A plasma level in ALL children compared to a healthy control group, 40 ALL children and 40 apparently healthy children as control were included in this part of the study. Thirteen out of 40 of the ALL children were newly diagnosed and 27 children were already on treatment; they were diagnosed by specialist physicians in the Oncology Unit in Basrah Children's Hospital. The ALL children included 21 males and 19 females and their ages ranged from 1 to 15 years. The control group composed of 40 apparently healthy children from a health care center matched for sex and age with the case group.

To compare VEGF-A plasma levels between standard risk group (SRG) and high-risk group (HRG) ALL children, the 40 patients were classified into SRG (n = 6) and HRG (n = 34) depending on oncologist estimation.

Samples collection: Three milliliters of venous blood were collected in ethylenediaminetetraacetic acid (EDTA) and the plasma was separated using centrifuge at 2000 rpm for 25 min. The obtained plasma samples were frozen at −20°C for subsequent analysis. Multiple freezing and thawing was avoided.

The exclusion criteria were children with other types of solid and hematological malignancy, hemoglobinopathies, bleeding disorder, previous injury, and recent surgery, which could further enhance angiogenesis.

Quantitative detection of VEGF-A: The analysis was completed according to the manufacturer's protocols of Human VEGF ELISA Kit – MyBioSource, Catalog No.: MBS355343, USA. The plasma level of VEGF-A in the study group was compared with that of the control.

***Statistical analysis*:**

Software package SPSS for Windows, version 22, was used to analyze the data. Mean and standard deviation were used for the variable with normal distribution while median was used to express the result of the variables which was different from normal distribution. *P*-value was assessed using Mann–Whitney U-test and considered statistically significant at < 0.05.

RESULTS

Table 1: General characteristics of the study population

Character	Case*		Control	
	n	%	n	%
1 – Age (Year)				
1–5	12	30%	12	**30.0%**
5–8	11	27.5%	18	**45%**
9–15	17	42.5%	10	**25%**
Total	40		40	
Mean + SD	**Mean = 13.3 ± 3.2**		**Mean = 13.6 ± 4**	
2 – Sex				
Male	21	52.5%	21	**52.5%**
Female	19	47.5%	19	**47.5%**
3 – Risk group				
****SRG**	6	15%		
*****HRG**	34	85%		
Total	40	100%		

Case: children with ALL. **SRG: standard risk group. ***HRG: high-risk group.

1 – VEGF-A plasma level in study groups

The mean of VEGF-A plasma concentration is higher in ALL patients as compared to control (Table 2). The difference was highly statistically significant (p < 0.001).

Table 2: VEGF-A plasma level in study groups

Study group	Median
Control (n = 40)	241.10
ALL children (n = 40)	1597.78

- Mann–Whitney U-test was used to make a comparison of the VEGF-A plasma level between control and ALL children.

Regarding risk groups, there is an association between the plasma level of VEGF-A and ALL risk groups (p = 0.01) with the highest levels observed in the HRG as shown in Table 3.

Table 3: The relationship of VEGF-A plasma level with ALL risk groups

Risk groups	Median
SRG (n = 6)	863.89
HRG (n = 34)	1835.11

- Mann–Whitney U-test was used to make a comparison of the VEGF-A plasma level between SRG and HRG.

DISCUSSION

Angiogenesis plays a central role in leukemia. Many factors stimulate angiogenesis, including VEGF-A. It is considered one of the most essential proangiogenic agents acting by paracrine and autocrine action and influencing leukemia cells to spread and survive. It is additionally a vascularization angiogenesis stimulator (8). Therefore many researchers performed the analysis of VEGF-A expressions in hematological malignancies (9). It has been suggested that children with ALL have an increased level of VEGF-A and microvascularity (10,11).

In this study, we found that the plasma level of VEGF-A was higher in ALL children than those in the control group. These results are consistent with Stachel et al. (2007) who described that the VEGF-A concentration in different hematological malignancies is considerably elevated (12). Moreover, Chand et al. found that the VEGF expression and micro vascular density (MVD) in bone marrow were augmented in hematological cancers including acute and chronic leukemia (13). The high level of plasma VEGF-A in ALL children could reflect the augmentation of angiogenesis in the bone marrow.

Our findings agree also with Zeng et al. who found the significantly increasing level of VEGF-A in different hematological malignancies including ALL (14). These outcomes might support the concept that leukemic cells induce angiogenesis.

Regarding the VEGF-A plasma level in ALL risk groups, we found that 6 out of 40 ALL patients were classified as SRG while 34 out of ALL patients were classified as HRG.

In our research, the plasma level of VEGF-A was lower in SRG than in HRG and the differences was statistically significant. This finding agrees with Agnieszka Mizia-Malarz who showed that the SRG participants with most favorable prognosis presented with the lowest VEGF-A, as compared to other groups with poorer prognosis (HRG). This may confirm the role of leukemic cells in VEGF-A production(8).

CONCLUSION

In this study, we found that the VEGF-A level increases in children with ALL compared to healthy children. Moreover, the VEGF-A plasma level in HRG was found to be significantly higher than that in SRG. These results could propose the role of leukemic cells in the production of VEGF-A. Further studies are recommended to identify the VEGF-A mechanism which might affect prediction, progression, and therapeutic effect of ALL.

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