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Vascular Endothelial Growth Factor-A Plasma Level and-460 *VEGF* Gene Single Nucleotide Polymorphism Significance in Childhood Acute Lymphoblastic Leukemia in Basrah, Iraq

Zainab A. Badr,[1]Wijdan N. AL-Moosawi,[2]SadiqK.Ali [3]

[1]B.Sc.pathological Analysis Teqniques, Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
[2]M.B.Ch.B; Ph.D, Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
[3]M.B.Ch.B,F.I.B.M.S, Department of Hematopathology, AL-Zahraa College of Medicine, University of Basrah, Basrah, Iraq
Correspondence: Zainab A .BadrEmail:zainabbadr5@yahoo.com

Abstract:

Background: Vascular Endothelial Growth Factor –A (VEGF-A) has a vital role in angiogenesis inhematological malignancies including acute lymphoblastic leukemia (ALL).Many singlenucleotide polymorphisms(SNPs) in *VEGF* gene may affect susceptibility of ALL in children.

Aim of the study: To investigate the frequency of -460 *VEGF* gene SNP and their impact on plasma level of VEGF-Ain children with ALL.

Material and method: This study included 40 children with ALL and 40 healthy children as controlcarriedout at the Oncology Unit in Basrah Children specialty Hospital from Oct 2019 to March 2020. VEGF-A plasma level evaluated using ELISA assay, PCRteqnique was used to detect the -460 VEGF SNP.

Result:VEGF-Aplasma level in ALLchildren was higherthan control, there is significant difference of -460 *VEGF*SNP between ALLchildren and healthy control and the impact of gene polymorphism on plasma level of VEGF-A in ALL children was highly significant.

Conclusion: VEGF-A level and gene SNPcould play a central role in ALL pathogenesisand progression.

Introduction:

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extra medullary sites(Ferri 2017).ALL account for approximately 33% of all malignancies among children aged bellow fifteen years(Belson, Kingsley, and Holmes 2007).

VEGF-A has a vital role in stimulating angiogenesis andincreasing vascular permeability, which reduced delivery of drug and metastasis of tumor cell inacute lymphoblastic leukemia(Yang et al. 2015).VEGF-A, when combined with their ligand-receptors, can promote the division of vascular endothelial cells and induce the generation of new vessels resulting in providing positive conditions for the occurrence and progression of the tumors(Han *et al*. 2016).

Gene polymorphism may display a crucial function in the malignant disorders prognosis and could clarify individual inconsistency in response to therapy(Chen *et al*. 2013)as a result, specific concentration has been focused on VEGF single-nucleotide polymorphisms (SNPs)such as +936C > T (rs3025039), −2578C > A (rs699947), −634G > C (rs2010963) and -460 T/C (rs833061) because they may change levels of VEGF expression(Liu *et al*. 2018).The link between *VEGF* geneSNP and risk for ALL in adults has been widely studied while, Few researchers have examined the relations between functional polymorphisms in *VEGF* and the prognosis of childhood ALL (Demacq *et al*. 2010).

This study aimed to investigate the frequency of -460T/C VEGF-A geneSNP and theirimpacton VEGF-A plasma levelin children withAcute lymphoblastic leukemia.

Material and method

A case-control study carried out from October 2019 toApril 2020. It was done on 40 children (21male, 19 female) with acute lymphoblastic leukemia who had been diagnosed by specialist physicians and attending the Oncology Unit in Basrah Children Specialty Hospital.Thirteen of patients (13/40) were newly diagnosed and twenty seven (27/40) were already on treatment. Their ages ranged from 1-15years old.

Control group healthy control group consisted of 40 healthy children from the health care center matched for age and sex with the study group.

Four milliliters of venous blood which were collected in EDTA (ethylenediaminetetraacetic acid) as anticoagulant from the study groups.

Serological study:2ml of the 4ml of collected samplewerecentrifuged at 3000 rpm for 15 minutes, plasma drown using a clean pipette into sterile plastic tubes and kept frozen at -20C till the serological examination was completed for detection the VEGF-A level. Multiple freezing and thawing was avoided. Plasma level of VEGF-A were evaluated according to manufacturer's protocols of ELISA kit (Human VEGF ELISA Kit-MyBioSource, Catalog No.: MBS355343, USA).

Molecular study:The other 2mlof4ml collected sample were used for total DNA extraction then used polymerase chain reaction(PCR) to detect *VEGF*-460T/C geneSNP.Total DNA had been extracted using ReliaPrep™ Blood GdnAMiniprep System Technical Manual System (Promega, USA. Cat. No: A5081, 100 Preps).

The selected *VEGF*–460T/C SNP was genotyped through conventional polymerase chain reaction (PCR). PCR analysis of gene polymorphism is based on the primers which was supplied by Alpha DNA (Canada). PrimersZarbock *et al*. (2009) :Sense primers(F1):5'-TGCGTGTGGGGTTGAGGGC-3',(F2):5'-CGTGTGGGGTTGAGGGT-3'and antisense primer: 5'-GGCTCTGCGGACGCTCAGTGA-3'(Zarbock et al. 2009).The thermal cycler conditions of PCR were:initial PCR at 95°C for 5 min, then denaturation at 94°C for 1 min, annealing at 60°C for 1 min (for40 cycles), Extension at 72°C for 1 min and the last extension at 72° C for 10 min.

To separate the PCR products, electrophoresis through a 2% agarose gel containing Ethidium Bromide was used. The *VEGF* -460T/C gene SNP at 341pb. For giving complete conformity of the results, the genotyping was repeated selecting randomly samples.

Statistical Analysis:

(SPSS) version 22 was used to analyze the data. Median and Mann-Witny U test used for quantitative non parametric data. Frequency, Chi-square and Exact Fisher test were used for qualitative data. P.value less than 0.05 wasapplied as statistically significant.

R**esult**

1- Plasma VEGF-A level in the study population:

Table (1) illustrates that the plasma level of VEGF-A is higher in ALL children than control. The difference was highly significant (p<0.001).

Table 1: Plasma VEGFA level in study groups:

Group	median
1- Study groups	
Control (n=40)	241.10
Case (n=40)	1597.78
P=0.0001 ** ** highly Significant at p<0.001	

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

2: Distribution of genotypes and frequency of alleles of the *VEGF* - 460 T/C SNP among study group:

The table2 illustrates that The genotype frequency of *VEGF* -460 T/C SNPs presented in 32/40 of the healthy control and in 24/40 of the ALL patients distributed between three genetic models, TT, TC, and CC. The -460T/C *VEGF-A* genotypes distribution between the case and control group was statistically differences (P<0.001). In our population, TT genotype was dominant in both control and patient. The TC, CC, and CC genotypes were higher in control than in the patient group.

Regarding -460T/C alleles, the T allele was detected in 27 of the control and in18 of ALL patients, while the C allele was detected in 19 of the control and 12 of the patient. The differences of both T and C allele frequencies was highly statistically significant among the study groups (*P<0.05*) with increase in control group.

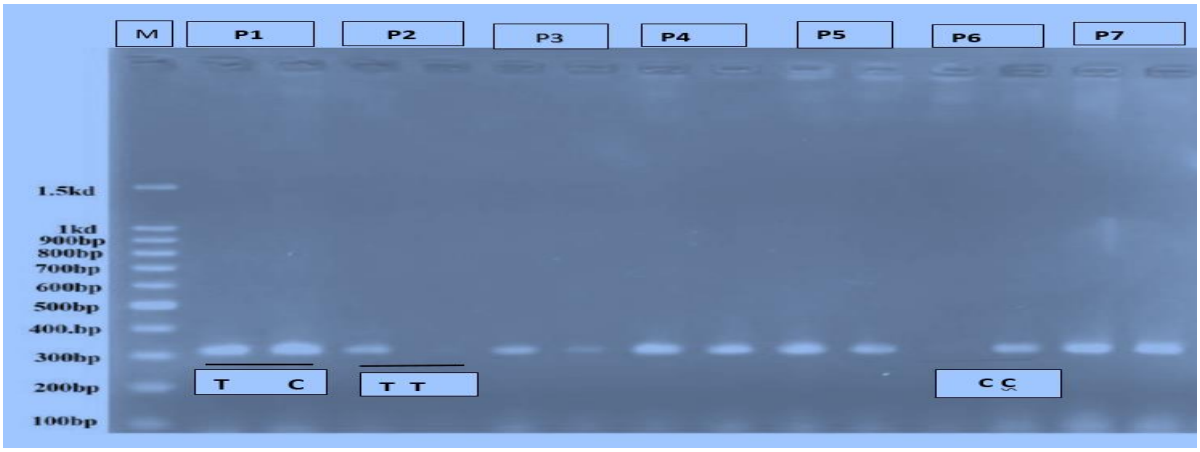

Figure 1:Agarose gel electrophoresis displays bands of PCR product of -460T/C *VEGF* SNP at 341 bp. M: ladder, P1, P3, P 4, P5, P7: heterozygous TC genotype, P2: homozygous TT genotype and P6: homozygous CC genotype.

Table 2: Genotype distribution of the *VEGF*- 460 T/C and frequency of allele among study groups

Control (n=32)Case (n=24)				
Genotypes	**n**	**n**	**OR(95%CIs)**	**P-value**

TT	14	12	0.87	0.000**
TC	13	8	1.29 (0.60-2.46)	0.000**
CC	5	4	0.93 (0.28-3.12)	0.008**
Allele frequency				
T	27	20	0.93	0.000**
C	18	12	1.1 (0.6-21.93)	0.000**

-OR: odd ratio, CIs: 95% confidence intervals.

-Fisher's test was used to assess p-value. ** Highly significant at p≤0.001.

3: Impact of -460T/C *VEGF* gene polymorphism on VEGF-A plasma level:

The relation between the VEGF plasma level and -460T/C Genotypes and Alleles among study groups was described in table 3, which shows that the association between-460T/*C VEGF* genotypes and alleles and its plasma levels in ALL children and control was statistically significant(P=0.0001).

Table 3: Correlation between VEGF plasma level and the -460T/C Genotypes and allelic frequency among study groups.

Genotypes		**Control**	**Case**	**p-value**
TT	**No.**	14	12	0.000**
	Median	344.39	1943.97	
TC	**No.**	13	8	0.000**
	Median	227.82	1561.69	
CC	**No.**	5	4	0.001**
	Median	249.03	1044.19	
Allelic frequency				
T	**No.**	27	20	0.000**
	Median	237.67	1860.03	
C	**No.**	18	12	0.000**
	Median	241.10	1259.50	

-Pearson correlation test was used.

** Highly significant at ($p \leq 0.001$).

Discussion

VEGF-A plasma level in the study population:

In this study using ELISA assay, we found that the plasma level of VEGF-A was significantly higher in patients with ALL than controls (P=0.0001). These findings are consistent with Zeng et al. (2014)who states that the level of VEGF-A is significantly elevated in various blood cancers (Zeng *et al*. 2014). The VEGF expression in bone marrow were measured by Chand et al, found out that the MVD and VEGF expression in BM were enhanced in various hematological cancers including in multiple myeloma, non- Hodgkin lymphoma, acute and chronic leukemia (Chand *et al*. 2016). The higher level of plasma VEGF in children with ALL than control could propose the angiogenesis intensification in the bone marrow, as a result of new plasmatic proliferation. Several studies have shown that VEGF-A production correlates to tumor proliferation and apoptosis; and support a potential role for VEGF in leukemia prognosis (Koomagi *et al*. 2001).

Allelic frequency and genotype distribution and of the *VEGF*- 460 T/C among the study group:

VEGF-460T/C SNPinvestigation shows that thegenotype of (TT) is more common among other genotypes in both ALL children and control group, with an enhancement in the occurrence of T allele compared to C allele.

Regarding the genotypes frequency within the leukemic group, we found that (TT) and (TC) genotypseare more frequent than (CC). This is consistent with the work done by Amal Mansour et al., 2015 who studied the link of three SNPs of VEGF (-2578C/A, -634G/C and -1154G/A) with the childhood ALLprognosis (Mansour *et al*. 2015). Studies show that the relationship between the *VEGF* polymorphisms and the risk of relapse, as a result, it could be a prognostic signal in childhood ALL (Demacq*et al*. 2010).

Association of plasma level of VEGF-A and *VEGF* -460 T/Cgenotypes:

In this study, we found that there was a significant effect of both genotypes (TT, TC, CC)and alleles (T,C)on the VEGF-A plasma level ($P<0.05$),so that *VEGF-A* polymorphisms may associate with VEGF expression levels.This result disagree with Mansour et al. who found that the association between the VEGF levels in ALL patients and different genotypes of both VEGF 460T/C and 1154 G/A SNPs, does not reach to statistical significance(Mansour *et al*. 2015)

Sample sizes, differences in ethnic backgrounds, population admixture, age, dissimilarities in treatment modalities and various selection factors may all result in to these discrepancies and conflicting findings.

Conclusion:

The level of VEGF-A and *VEGF* gene SNP could play acritical role in ALL severity, prognosis survival and clinical outcomes of drug treatment so this field is quite interesting and can open new avenue in the future. Further studies with large number of populationare recommended.

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