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

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Abstract:

Background: Vascular Endothelial Growth Factor –A (VEGF-A) has a vital role in angiogenesis in hematological malignancies including acute lymphoblastic leukemia (ALL). Many single nucleotide 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-A in children with ALL.

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

Result: VEGF-A plasma level in ALL children was higher than control, there is significant difference of -460 VEGF SNP between ALL children 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 SNP could play a central role in ALL pathogenesis and 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 and increasing vascular permeability, which reduced delivery of drug and metastasis of tumor cell in acute 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 gene SNP 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 gene SNP and their impact on VEGF-A plasma level in children with acute lymphoblastic leukemia.

**Material and method**

A case-control study carried out from October 2019 to April 2020. It was done on 40 children (21 male, 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 1-15 years 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 sample was centrifuged at 3000 rpm for 15 minutes, plasma drawn using a clean pipette into sterile plastic tubes and kept frozen at -20°C 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 2ml of 4ml collected sample were used for total DNA extraction then used polymerase chain reaction (PCR) to detect VEGF–460T/C gene SNP. 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). Primers Zarbock et al. (2009): Sense primers (F1): 5’-TGCGTGTGGGGTTGAGGGC-3’, (F2): 5’-CGTGTGGGGTTGAGGGT-3’ and antisense primer: 5’-GGCTCTGCGACGCTCAGTGGA-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 (for 40 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–Witnuy 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 was applied as statistically significant.

**Result**

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 VEGF-A level in study groups:**

<table>
<thead>
<tr>
<th>Group</th>
<th>median</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Study groups</td>
<td></td>
</tr>
<tr>
<td>Control (n=40)</td>
<td>241.10</td>
</tr>
<tr>
<td>Case (n=40)</td>
<td>1597.78</td>
</tr>
<tr>
<td><strong>P=0.0001</strong> ** highly Significant at p&lt;0.001**</td>
<td><strong>highly Significant at p&lt;0.001</strong></td>
</tr>
</tbody>
</table>
- 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 table 2 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 in 18 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.

![Agarose gel electrophoresis](image)

**Figure 1:** Agarose gel electrophoresis displays bands of PCR product of -460T/C VEGF SNP at 341 bp. M: ladder, P1, P3, P4, 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

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control (n=32)</th>
<th>Case (n=24)</th>
<th>OR (95% CIs)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>n</td>
<td>n</td>
<td>OR(95% CIs)</td>
<td>P-value</td>
</tr>
<tr>
<td>TC</td>
<td>n</td>
<td>n</td>
<td>OR(95% CIs)</td>
<td>P-value</td>
</tr>
<tr>
<td>CC</td>
<td>n</td>
<td>n</td>
<td>OR(95% CIs)</td>
<td>P-value</td>
</tr>
</tbody>
</table>
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.

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

- OR: odd ratio, CIs: 95% confidence intervals.
- Fisher’s test was used to assess p-value. ** Highly significant at p≤0.001.

-Pearson correlation test was used.
** Highly significant at (p≤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 SNP investigation shows that the genotype 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) genotypes are 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 ALL prognosis (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/C genotypes:**

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 a critical 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 population are recommended.

**References**


Zeng, Dongfeng, Jinliang Wang, Peiyan Kong, Cheng Chang, Jieping Li, and Jiali Li. 2014. “Ginsenoside Rg3 Inhibits HIF-1α and VEGF Expression in Patient with Acute Leukemia via Inhibiting the Activation of PI3K/Akt and ERK1/2 Pathways.” *International Journal of Clinical and Experimental Pathology* 7(5):2172.