Investigation the Association of the VEGFR-2 and -2578C\A Polymorphism as a Risk Factor for Incidence of Lung Cancer in Babylon Province

Ali K. Dahir[1], Alaa S. Al-Awad[2], Moaed E. Al-Gazally[3]

[1]College of Medicine, Babylon University, Iraq, [2]Prof. Dr., Oncology Center, Merjan Teaching Hospital, Babylon, Iraq, [3]Prof. Dr., College of Medicine, Babylon University, Work Now at University of Al-Ameed, Iraq

Abstract

Background: VEGF gene polymorphisms can induce either increase or inhibition of VEGF secretion, with altered promoter activity.

Aim: We investigated the association of the VEGFR-2 and -2578C\A polymorphism in the VEGF gene with Lung cancer risk in Babylon province. Methodology: VEGFR-2 level was measured by Enzyme Linked Immunoabsorbant Assay ELISA and Genotyping of the VEGF-gene variation (-2578A\C) was performed using the amplification refractory mutation system PCR. We investigated the association of VEGF gene variants with different clinicopathological features of lung cancer patients.

Results: No significant difference was seen of the VEGFR-2 levels in lung cancer patients when compared with control group (P=092), the allelic frequency of -2578 A\C VEGF gene no difference was seen in patients lung cancer (p=0.652). Hardy-Weinberg equilibrium showed a significant difference cases group (p=0.016), also revealed A allele exhibits a pathological behavior and it appears in over-dominant model (p=0.05), this proves that there is significant association of -2578A\C VEGF gene in lung cancer patients.

Conclusion: Our results showed no significant association of the VEGFR-2 in lung cancer patients while showed a significant association of the VEGF -2578C\A polymorphism with LC susceptibility in Babylon province .The VEGF -2578C\A heterozygote significantly increases the risk and can be useful as a predisposing genetic marker.

Keywords: *Lung cancer, VEGFR-2, polymorphism, dominant model.*

Introduction

Lung cancer (LC) is the main cause of cancer-related deaths worldwide, it is a complex and highly heterogeneous disease[1]. About two million person are diagnosed with lung carcinoma each year and most of them diagnosed at an advanced stage[2]. Iraqi cancer broad reports that Lung cancer is the second most common cancer in the Iraq province[3].The lack of effective treatment choice and high mortality make lung cancer a major public health challenge all over the world[4]. With the advent of next-generation genotyping and in-depth understanding of the molecular biology of lung cancer, genotyping of single-nucleotide polymorphisms (SNPs)may be pivotal in the personalized treatment for patients with lung cancer[5]. Angiogenesis it is known as complex process of the formation of new networks of blood vessels. Angiogenesis has amajor role in tumor progression and metastasis[6]. VEGF plays a main role in the progress and prognosis of malignancy[7]. VEGF is an important in establishinga vascular supply within the tumorin lung carcinoma[8]. The VEGF/VEGF-receptor axis is composed of multiple ligands and receptors with overlapping and distinct ligand–receptor binding

Corresponding Author:
Ali K. Dahir
Lecturer,Faculty of Medicine, Babylon University, Iraq
e-mail: alidahir@yahoo.com

specificities, cell-type expression, and function[9]. VEGFR-2 is a type V receptor tyrosine kinase mainly known to be expressed in vascular endothelial cells and encoded by theKDR gene[10]. This receptor responds to the signal of VEGF binding, which initiates a phosphorylation cascade that ultimately involves nuclear regulatory targets resulting in enhancement of endothelial proliferation, angiogenesis and cell migration[11].The VEGF gene is located at chromosome 6p21.3, covering14 kb in length with 8 exons and 7 introns [12].Being highly polymorphic, about 30 single SNPs have beenidentified and described[13].Several SNPs of VEGF havebeenreported to be associated with individual susceptibilityto cancer and can alter the VEGF expression and proteinproduction[14-20].

Materials and Method

Determination of Serum VEGFR-2 Concentration: The VEGFR-2 concentration is measured by enzyme linked immune- absorbent assay kit (Sandwich-ELISA) type as the method[20].

DNA Extraction: DNA were extracted by the procedure depending on manufacture protocol and was detected by using agarose gel electrophoresis technique[15].

VEGF -2578C/A (rs699947) genotyping: VEGF -2578C/A genotyping was detected by using amplification-refractory mutation system –PCR(ARMS-PCR). ARM Systems are based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. Following an ARMS reaction the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele. The VEGF -2578C\A genotyping primers were designed by using primer3 software as showed intable(1)[25].The ARMS-PCR done in a reaction volume of 20µL containing $MgCL_2$ (0.5µL), FO- 1µL, RO- 1µL, RI- 1µL, RI-1µL of 10pmol of each primers and 8µL from Master Mixof2.5X(Cyntol, Russian). The final volume of 18µLhas been modified by adding nuclease free ddH_2O(5.5 µL).Finally, the DNA template (2µL), was added from each cases.

Amplification conditions of VEGF genotyping: The amplification conditions used were at 94 C for 5 minutes followed by 35 cycles of 94C for 35sec, 63 C for 40 sec, 72 C for 45 sec followed by the final extension at 72 C for 5minutes. The amplification products were separated by electrophoresis through 2% agarose gel stained with 3µL ethidium bromide and visualized on a UV transilluminator. Primers FO and RO flank the exon of the VEGF -2578C\A gene, resulting a band of 353bp to act as a control for DNA quality and quantity. Primers FI and RO amplify a wild-type allele (C allele), generating a band of 229 bp, and primers FO and RI generate a band of 149bp from the mutant allele(A allele),.The best temperature was determined to be 58°C in the temperature range of 55°C to 63°C tested with a gradient PCR thermocycler. The annealing temperature was lowered from 60 to 58°C to favor the binding of both forward wild and reverse mutant primers that contain mismatches to the templates. The number of cycles was increased from 30 to 35 cycles, significantly enhancing the yields of all three PCR products. Together, these changes resulted in a more robust amplification of the mutant allele and a less competing reaction from the control, as shown by the relative intensities of the corresponding bands on agarose gel electrophoresis.

Results

The present results on patients showed that high age frequency of cancer occurred between (45-80) years old.The mean and standard deviation of age for lung cancerpatients when compared with control group, as showed in table(2).The current results on patients showed that high age frequency of cancer occurred between (45-80) years old, this due to the lung cancer incidence is very low before age 25 years and increases with increasing age up to 40 years due to several causes such as environmental factors, the nutrition, poor health education, radiation exposure, smoking, repeated injuries, and previous lung disease[16].The current results agree with many studies in Iraq performed on lung cancer[17-19], they expected that the risk of lung cancer is higher in middle age and elderly Men and women than in younger, this risk increase as a woman and Men ages, rising sharply after the age of 50 years old**.**

VEGFR-2 concentration in the serum: This results showed no significant difference for VEGFR-2 serum concentration in lung cancer patients when compared with control groups, P-value=0.92,as shown in the table(3).

Genotyping of the VEGF gene variation(-2578A\C)rs699947: The Hardy-Weinberg Equilibrium Analysis: The genotype distributions and allele frequencies of the SNPs -2578C\A located in the VEGF gene showed a deviation from HWE(P=0.016)in the

patients group while thegenotype distributions and allele frequencies of the SNPs-2578C\Ashowed no deviation from HWE (p=1) in the control group.

Study population: All demographic characteristic of the subjects are showed as follows a total of 45Lung cancer patients and the same number of gender matched healthy control wereanalyzed. This research was confirmed by the Research ethics committee, University of Babylon collage of medicine. Blood(5ml) samples were collected from participants in EDTA and gel tubes.

The VEGFR-2 level in different genotype individual of the -2578 C\A VEGF gene: This study has shown no association of the VEGFR-2 levels in different individual that carry different genotype of the -2578C\AVEGF gene. P-values>0.05.

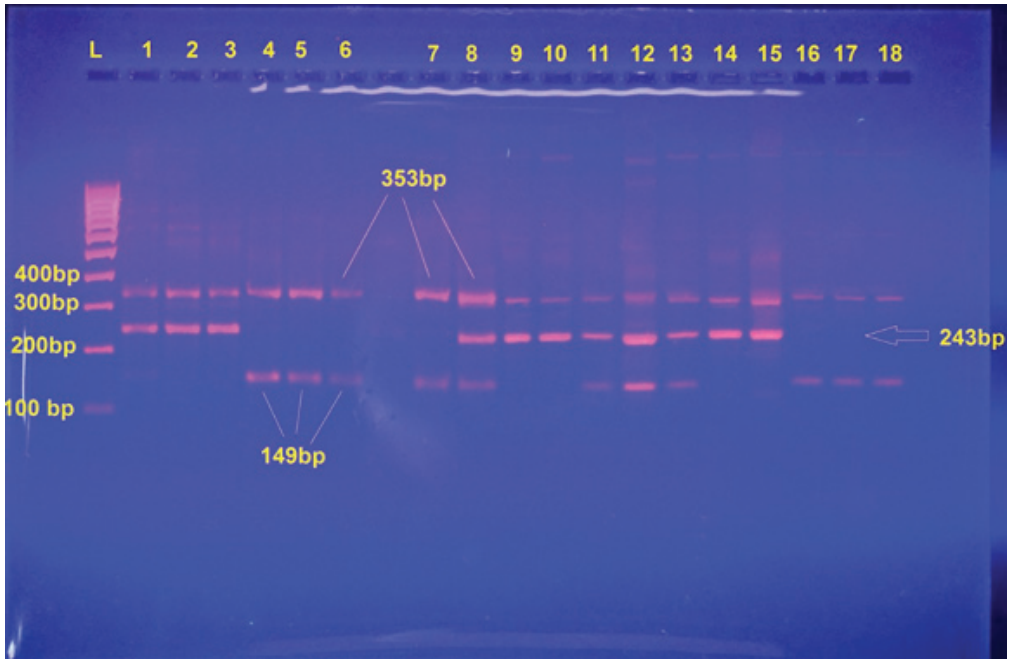
Discussion

Several studies detect a significant increase in VEGFR-2 in Lung cancer patients as a result of angiogenesis process because of the increased need for oxygen. This study did not show the significant difference in the VEGFR-2 levels of the two groups,and this does notagree with [20-22], that revealed a significant difference in regards to VEGFR-2 levels in lung cancer patients when compared to control groups. VEGF plays a paramount role in an angiogenesis through different mechanisms[23]. High VEGF expression is associated with tumor growth and metastatic process, while the inhibited VEGF expression results in suppressed growth of cancer[24]. Several functional polymorphisms of the VEGF gene that may affect serum VEGF expression level, included −634G\C, −1154G\A, 936C\T, −1498C\T, −2578C\A, and −460C\T [25].The −2578C/A of VEGF gene, have been demonstrated to modulate the levels of VEGF expression in tumor growth especially with lung cancer[26].Recently, the effects of the VEGF gene polymorphism on the Lung cancer risk have been extensively studied; however, the results of these studies were conflicting and ambiguous.The findings of our study proposed that VEGF -2578C\A variant significantly increased the risk of Lung cancer.These results indicate that relative risk of lung cancer associated with −2578C/A of the VEGFgene lung carcinoma in their patients.The present results agreement with[27-29], all of which demonstrated a high significant correlation of the-2578C\A VEGF with lung carcinomas, and not agreement with Liu C et al 2015 and Yang F et al 2018[30] these studies were conducted on Asians, Caucasians and Indians it did not show a significant association of the -2578C\A VEGF gene and lung cancer.In conclusion, our result showed no significant correlation with regards to levels of VEGFR-2 in lung cancer patients while showed a significant associations of VEGF -2578C\A polymorphism with LC susceptibility in Babylon population. VEGF -2578C\A in over dominant model significantly increases the risk of Lung cancer and can be useful as predisposing genetic marker for LC.

Table(1): Amplification-Refractory Mutation System–PCR Primers for VEGF-2578C\A Gene Polymorphism

Direction		Primer Sequence	AT	Product size
FO-VEGF		5-CCTTTTCCTCATAAGGGCCTTAG-3	58 C	353bp
RO-VEGF		5-AGGAAGCAGCTTGGAAAAATTC-3		
FI–VEGF	A allele	5-TAGGCCAGACCCTGGCAA-3		149bp
RI-VEGF	C allele	5-GTCTGATTATCCACCCAGATCG-3		243bp

Fo-outer forward primer, Ro-Reverse outer primer; AT-annealing temperature; FI-Inner forward primer, RI-Inner Reverse primer



Figure(1): Genotyping of VEGF gene polymorphism rs699947 of gene by PCR-ARMS technique, lanes 4,5,6,7,16,17 and 18 AA genotype; lanes 1,2,3,9,10,14 and 15 CC genotype; lanes 8,11,12 and 13 AC genotype.

Table(2): Genotype exact test for Hardy-Weinberg equilibrium, P-value of Chi square

Parameter	C/C	A/C	A/A	C	A	P-value
All subjects	24(27%)	53(59%)	13(14%)	101	79	0.088
Control	15(33%)	22(49%)	8(18%)	52	38	1
Case	9(20%)	31(69%)	5(11%)	49	41	0.016

Table(3): Allele Frequency, Odd Ratio and P-value Between Patient and Control inall sample

	Control		Case		OR (95% CI)	P-value*
Allele	Count	Proportion	Count	Proportion		
C	52	0.58	49	0.54	0.87(0.48-1.57)	0.652
A	38	0.42	41	0.46	1.14(0.63-2.06)	

Table(4): VEGF(-2578C\A) genotype association with LC under different models of inheritance

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	C/C	15 (33.3%)	9 (20%)	1.00	0.15
	A/C	22 (48.9%)	31 (68.9%)	2.35 (0.87-6.32)	
	A/A	8 (17.8%)	5 (11.1%)	1.04 (0.26-4.18)	
Dominant	C/C	15 (33.3%)	9 (20%)	1.00	0.15
	A/C-A/A	30 (66.7%)	36 (80%)	2.00 (0.77-5.21)	
Recessive	C/C-A/C	37 (82.2%)	40 (88.9%)	1.00	0.37
	A/A	8 (17.8%)	5 (11.1%)	0.58 (0.17-1.93)	
Over dominant	C/C-A/A	23 (51.1%)	14 (31.1%)	1.00	0.05
	A/C	22 (48.9%)	31 (68.9%)	2.31 (0.98-5.47)	

Table(5): Mean, Std.Error, Std.Dev., and P-value of VEGFR-2 serum level in different genotype person

(I) Genotype	Mean	St. Deviation	(J) Genotype	Mean Difference (I-J)	Std. Error	p-value
A/A(13)	2613.38	841.39	A/C	175.32	329.60	0.59
			C/C	161.50	366.04	0.66
A/C(52)	2438.05	1131.46	A/A	-175.32	329.60	0.59
			C/C	-13.81	262.30	0.95
C/C(24)	2451.87	1008.27	A/A	-161.50	366.04	0.66
			A/C	13.81	262.30	0.95

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

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