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Original Research Article

VEGF mRNA Expression in Epithelial Ovarian Cancer: Correlation with rs699947 Gene Variant

Yuda Nabella Prameswari[1*], Dwi Anita Suryandari[2], Dewi Sukmawati[3], Luluk Yunaini[2], Ria Kodariah[4]

[1]*Department of Medical Biology, Faculty of Medicine and Health Science, Universitas Sultan Ageng Tirtayasa, Indonesia*
[2]*Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Indonesia*
[3]*Department of Histology, Faculty of Medicine, Universitas Indonesia, Indonesia*
[4]*Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Indonesia*

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Abstract

Background: Angiogenesis is the formation of new blood vessels, is crucial for cancer growth and metastasis, including in epithelial ovarian cancer (EOC). Vascular Endothelial Growth Factor (VEGF) regulates angiogenesis, and its elevated mRNA expression is linked to poor prognosis in cancer. Genetic variations, such as the rs699947 polymorphism in the VEGF gene, can affect VEGF expression and contribute to cancer progression.**Objective**: The primary aim of this study is to examine the distribution of the VEGF rs699947 polymorphism and its correlation with VEGF mRNA expression levels in patients with low-grade and high-grade EOC at Dr. Cipto Mangunkusumo Hospital, Indonesia.**Methods**: This research is a cross-sectional analysis involving 65 normal female whole blood samples and a total of 80 ovarian cancer biopsy samples, including 15 ovarian cysts as expression calibrators, along with 36 low-grade and 29 high-grade EOC samples. The distribution of genotypes and alleles of the VEGF rs699947 polymorphism was assessed through ARMS PCR analysis, while VEGF mRNA expression was quantified using real-time qPCR. **Results**: Significant differences were observed in both genotype (p<0,01) and allele (p=0,000) distributions between the normal and cases group. The relative mRNA expression of VEGF was significantly elevated in both low-grade and high-grade EOC. Individuals with the homozygous VEGF rs699947 AA genotype exhibited the highest mRNA expression compared to other genotypes. In contrast, individuals carrying the CC genotype showed the lowest correlation with VEGF mRNA expression in both low-grade and high-grade EOC. **Conclusion**: This study shows that the A allele of VEGF rs699947 is correlated with increased VEGF mRNA expression in EOC patients, particularly in those with the AA genotype. Conversely, the C allele may offer a protective effect against EOC, as the CC genotype is linked to lower VEGF mRNA expression. Genetic screening for VEGF rs699947 could facilitate early detection and inform targeted therapeutic strategies.

Keywords: *Angiogenesis; Epithelial Ovarian Cancer; rs699947; Single Nucleotide Polymorphism; VEGF*
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INTRODUCTION

Ovarian cancer is one of the most prevalent malignancies in gynecology affecting women. According to data from the World Health Organization's International Agency for Research on Cancer (IARC) and the Global Burden of Cancer Study (GLOBOCAN), there were 324,603 new cases of ovarian cancer worldwide in 2022.[1] Additionally, ovarian cancer ranked as the fourteenth leading cause of cancer-related mortality, with 206,956 reported fatalities.

*Corresponding author:
E-mail: *dr.yudanabellap@gmail.com*
(Yuda Nabella Prameswari)

In Indonesia, the number of newly diagnosed ovarian cancer cases in the same year reached 15,130, making it the third leading cause of cancer-related mortality among women, with a total of 9,673 fatalities.[1] Furthermore, data from 2020, sourced from Dharmais Cancer Hospital, reported that ovarian cancer accounted for 3.91% of the total 45,422 cancer cases recorded.[2] Approximately 90% of ovarian cancer cases are of the epithelial type, originating from the invagination of the ovarian surface epithelium. EOC is classified into low-grade and high-grade based on its progression, molecular characteristics, and histological type.[3] In general, low-grade EOC is associated with a more vorable prognosis, characterized by slower progression and a lower rate of proliferation compared to high-grade EOC.[4] Additionally, over 60% of patients are diagnosed at a clinically advanced stage due to the rapid and asymptomatic growth of cancer cells.[4]

Cancer is defined by specific traits known as the hallmarks of cancer. One of these hallmarks in solid tumors is increased angiogenesis, which is referred to as the development of new blood vessels from existing ones.[5] Angiogenesis has been identified as a vital component in tumor growth and metastasis. Tumor cells activate an imbalance between pro-angiogenic and anti-angiogenic factors, regulating the angiogenesis process through the angiogenic switch.[6] This mechanism stimulates endothelial cells to undergo proliferation, migration, invasion, and adhesion, leading to the formation of new blood vessels.[7] One of the key pro-angiogenic molecules that serves as a major mediator of cancer angiogenesis is VEGF.[6]

The VEGF gene belongs to the growth factor family, located on the short arm of chromosome 6 (6p12-p21), and plays a crucial role in stimulating angiogenesis and increasing microvascular permeability through interactions with specific receptors expressed on vascular endothelial cells.[8] When VEGF binds to its receptor, relay proteins are activated, transmitting signals into the endothelial cell nucleus. This process induces endothelial cells to produce MMPs **(Matrix Metalloproteinases)**, enzymes responsible for breaking down the extracellular matrix, including proteins and polysaccharides in the intercellular space.[9] Once the extracellular matrix is degraded, endothelial cells can migrate into the surrounding tissue. Subsequently, endothelial cells undergo proliferation, gradually transforming the hollow space into mature blood vessel structures with the assistance of adhesion factors such as **integrin α or β.[10]**

Single nucleotide polymorphisms (SNPs) are a type of genetic variation in the human genome that play a role in regulating **cell cycle control, DNA mismatch repair and metabolism** and are linked to an increased genetic susceptibility to cancer.[11] SNPs are located in multiple genetic regions, such as **promoters, exons, introns, as well as the 5′-UTR and 3′-UTR**. As a result, variation in gene expression and their effects on cancer susceptibility may vary depending on the specific location of the SNP.[12] Promoter-associated polymorphisms can influence gene expression by modifying **promoter activity, the binding of transcription factor, mRNA stability, translation, DNA methylation, and histone modification.[13]** In the **VEGF** gene, one of the polymorphisms located in the promoter region is **rs699947 (-2578C/A).** This polymorphism has been associated with an increased risk of developing **ovarian[14] and breast cancer** in Asian populations.[15] A number of studies have been performed to investigate the relationship between **VEGF rs699947** and ovarian cancer, including research carried out by scientists in **China and India.[16] Thus far,** studies investigating the association between the VEGF rs699947 genetic variation and ovarian cancer based on its histopathological subtypes remain highly limited. Therefore, this study aims to analyze the VEGF rs699947 genetic variation and its mRNA expression in EOC.

MATERIALS AND METHODS

This study is designed as a cross-sectional analysis, with the aim of examining the correlation between genetic variants and VEGF mRNA expression and their association with the risk of low-grade and high-grade EOC. Given that VEGF plays a crucial role in the development and progression of cancer, we hypothesize that VEGF mRNA expression is elevated in both low-grade and high-grade EOC, and that individuals carrying the rs699947 genetic variant are at an increased risk of developing EOC.

The primary samples utilized in this study were ovarian cancer biopsies obtained from women who underwent surgery at RSUD Dr. Cipto Mangunkusumo between 2016 and 2021. The samples were obtained from the Biobank Research IMERI-FKUI and the Department of Anatomical Pathology FKUI in the form of formalin-fixed paraffin-embedded (FFPE) blocks. To determine the minimum sample size required for this study, a proportional formula calculation was performed, resulting in a minimum sample size of 28 samples. A total of 280 ovarian biopsy blocks were available for the study. However, only 65 blocks met the criteria for epithelial ovarian cancer and were selected for analysis. These 80 ovarian biopsy blocks were used in the study and categorized into three groups: 15 ovarian cysts as expression calibrators, 36 low-grade EOC and 29 high-grade EOC. This study also involved 65 whole blood samples from healthy women who donated blood at the Indonesian Red Cross in Central Jakarta, aged between 40 and 70 years. The participants had no personal history of cancer, ovarian cysts, endometriosis, PCOS, and had no familial history of cancer over the past three generations. Blood samples from women meeting these criteria were used as the normal control group for genotype analysis and rs699947 allele distribution. This study received approval from the Medical Research Ethics Committee of the Universitas Indonesia (No. KET-689/UN2.F1/ETIK/PPM.00.02/2022), and all participants were provided with information regarding the purpose of the study as the normal control group. Informed consent was signed and obtained from all participants who agreed to take part in the study prior to their involvement.

The study to analyse the genotype utilized two types of DNA samples were used: whole blood for the normal subjects and FFPE biopsies for the cases. DNA was isolated from whole blood using the salting-out technique. This process starts with the lysis of red blood cells using red blood cell lysis solution (RBC), followed

by the lysis of cells and nuclei using a cell lysis solution containing Tris HCl 1M, EDTA 0.5M, and 10% SDS, as well as protein precipitation using 5M ammonium acetate. For the extraction of DNA and RNA from FFPE ovarian biopsy samples, paraffin blocks were cut to a thickness of 6x5 µm, and then microdissection was performed to separate cancerous tissue from other tissues. The samples were subsequently deparaffinized with xylene to dissolve the paraffin, and then rehydrated using ethanol. Following the deparaffinization process, the samples were further processed using the gSYNC™ DNA Extraction Kit protocol. The DNA purity was assessed using a NanoDrop spectrophotometer (Maestrogen) at a wavelength of 260/280nm, with DNA extracted from these blocks expected to have a purity ratio between 1,7–1,9.

VEGF rs699947 was identified using T-Arms PCR with primers designed uthrough the Primer1 application, accessible at http://primer1.soton.ac.uk. The reference sequence used was sourced from the NCBI GenBank Database. The primers were subsequently analyzed using BLAST (NCBI) to evaluate their specificity. The expected PCR product size was less than 200 bp due to the degraded nature of the DNA extracted from FFPE blocks. Once the primers were obtained, T-ARMS PCR optimization was carried out to identify the optimal conditions of PCR. Amplification was carried out using three separate tubes, each containing a set of primers was used: forward outer and reverse outer for the internal control, forward outer and reverse inner for the alternative allele, forward inner and reverse outer for the wild-type allele. The master mix (25 µL) contained 12,5 µL of MyTaq™ HS Red Mix (Bioline) 2x, 1 µL each of forward and reverse primers, 100 ng of DNA template, and water free of nuclease. DNA samples were processed through 35 amplification cycles, starting with a pre-denaturation step at 94°C for 5 minutes. Next, denaturation was performed at 94°C for 30 seconds, annealing at 63°C for 30 seconds, and elongation at 72°C for 30 seconds. The extension time was prolonged at 72°C for 7 minutes at the end of the cycles. The amplified DNA molecules were separated based on their size using gel electrophoresis. The amplification products were pooled into a single tube and visualized through agarose gel electrophoresis at 3%, dissolved in 50 ml of TAE buffer. Bioline HyperLadder™ (Bioline) 50bp was used as a DNA marker. Finally, the bands formed after electrophoresis were visualized using an ultraviolet (UV) illuminator with a UV longlife™ filter from Spectroline.

RNA was extracted from paraffin-embedded samples following the protocol of the Quick-RNA Miniprep Plus Kit (ZymoResearch) for FFPE. A total of 100 ng of RNA was then converted into cDNA using the ReverTra Ace™ qPCR RT Master Mix with gDNA Remover kit (Toyobo). The resulting cDNA template was used for qPCR. VEGF gene expression was assessed relative to GAPDH gene expression. The PCR master mix was prepared on ice and made according to the manufacturer's instructions using SensiFAST SYBR Lo-ROX Mix® (Meridian Bioscience). Primers for VEGF were designed using the Primer Quest™ Tool (IDT DNA), with the primer sequences listed in Table 2. Amplification was performed using the 7500 Fast Real-Time PCR System, with two replicates for each sample. The relative VEGF expression values were qualitatively categorized based on the cycle threshold (Ct) values obtained: <20 (high expression), 20–25 (high), 25–30 (moderate), 30–35 (weak), and 35–40 (very weak). The relative expression level of the gene will be compared with the internal control, calculated using the Livak method.

Table 1. Primers used in ARMS PCR

Primer	Sequence (5' → 3')	TM (°C)	GC (%)	Amplicon size (bp)
VEGF (rs699947)				
Forward Outer	AAATTGAGGGAAATTGCTGCATTCCCATTC	65	40	
Reverse Outer	GAACAAAGTTGGGGCTCTGAGGCCTG	67	57	150
Forward Inner (A allele)	GCCAGCTGTAGGCCAGACCCTGGTAA	69	61	94
Reverse Inner (C allele)	ACCAGTCAGTCTGATTATCCACCCAGACCG	68	53	111

Table 2. Primer used in qPCR

Primer	Sequence (5' → 3')	Amplicon size
VEGF		
Forward	CTGTCTAATGC CCTGGAGCC	94 bp
Reverse	ACACGTCTGC GGATCTTGTA	

Table 3. Histological Types of EOC in the Study Subjects

Histological types	Total cases	Percentage (%)
Low-grade		
Low-grade Serous Carcinoma	2	(5,56)
Mucinous Carcinoma	9	(25)
Clear Cell Carcinoma	11	(30,56)
Endometrioid Carcinoma	14	(38,89)
High-grade		
Carcinosarcoma	2	(6,9)
High-grade Serous Carcinoma	27	(93,1)

Table 4. Genotype and Alleles Frequencies of rs699947 in the VEGF

rs699947	Frequency (%)		[a]*p*-value	[b]OR ([c]95% CI)
	Normal (n = 65)	Case (n = 65)		
Genotype				
CC	58,5	33,8	0,005*	0,364 (0,178-0,741)
CA	26,2	4,6	0,001*	0,137 (0,038-0,493)
AA	15,4	61,5	0,000*	8,800 (3,803-20,361)
Allele				
C	71,5	36,2	0,000*	0,225 (0,134-0,380)
A	28,5	63,8	0,000*	4,439 (2,632-7,486)

Note: a = Chi-square test, b = Odd ratio, c = 95% confidence interval, * = $p < 0,01$

Table 5. HWE Analysis of rs699947 in the VEGF gene

rs699947	Normal		*p*-value	Case		*p*-value
	Expected	Observed		Expected	Observed	
Genotype						
CC	33,27	38		8,5	22	
CA	26,47	17	0,015	30,01	3	0,000
AA	5,27	10		26,5	40	

Chi-square analysis was conducted to evaluate the correlation between genotype and Fisher's exact test was applied to examine allelic frequency distributions within the EOC groups. Differences in the relative mRNA expression levels of VEGF across groups were analyzed using the non-parametric Kruskal-Wallis test due to the non-normal distribution of the data. The correlation between genotype and relative mRNA expression of VEGF was assessed through Spearman's correlation test. Statistical analyses were conducted using SPSS version 25, with a p-value of less than 0.05 considered statistically significant.

RESULTS

In this study, endometrioid carcinoma was the most common histological type in the low-grade EOC group, whereas the high-grade EOC group was primarily composed of High-grade Serous Carcinoma (HGSC), as shown in Table 3. The mean age of participants at the time of diagnosis for low-grade EOC was 51,61 years, while for the high-grade EOC group, it was 52,51 years. In comparison, the mean age of subjects in the healthy normal group was 51,1 years.

According to the Chi-square test results shown in Table 4, there was a significant difference in the genotype frequency ($p < 0,01$) and allele type ($p = 0,000$) of VEGF rs699947. The AA genotype was found in 61,5% of the case group and showed an odds ratio (OR) greater than 1, indicating that individuals carrying the AA genotype are 8,8 times more likely to develop ovarian cancer, with a 95% confidence interval (CI) of 3,803–20,361, compared to the CC and CA genotypes. Allele A, as the alternate allele, had an allele frequency of 28,5% in the normal group and 63,8% in the case group. Additionally, allele A showed an OR greater than 1, suggesting that individuals with allele A have a 4,439 times higher likelihood of developing ovarian cancer, with a 95% confidence interval of 2,632–7,486, compared to allele C, which is protective against ovarian cancer as indicated by an OR less than 1. In contrast allele C, as the reference allele (wild type), showed a protective effect, yielding an odds ratio of 0,225 (95% CI 0,134–0,380).

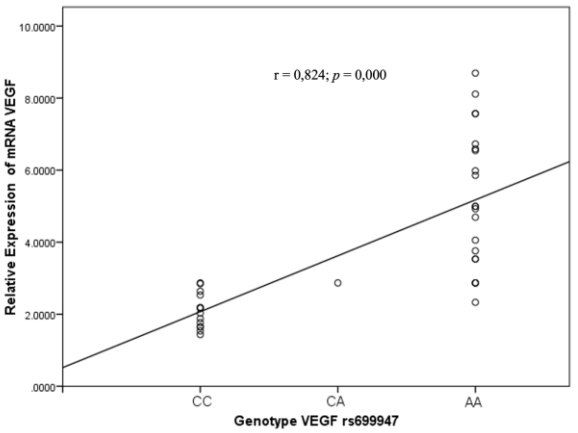

Figure 1. Correlation between the VEGF rs699947 genotype and VEGF mRNA expression levels in low-grade EOC

The data presented in Table 5 indicates that the distribution of the VEGF rs699947 genotype in both the normal and case groups is not consistent with Hardy-Weinberg equilibrium, as the HWE p-value is <0.05. This suggests that the genotype frequencies in this sample are not stable or are not in equilibrium from one generation to the next.

The relative mRNA expression of VEGF obtained is the result of calculations using the Livak method, comparing the RNA expression levels in the case group with ovarian cysts as the calibrator. The Kruskal-Wallis test was used for the statistical analysis to determine the relative mRNA expression of VEGF. The results showed a significant difference in the relative mRNA expression of VEGF across the ovarian cyst, low-grade, and high-grade EOC groups, with a *p*-value of 0,000. The relative mRNA expression of VEGF in low-grade EOC (3,92 ± 2,12) was significantly higher compared to the ovarian cyst group (1,10 ± 0,51). Furthermore, high-grade EOC showed a significantly greater VEGF mRNA expression (12,52 ± 3,34) when compared to both low-grade EOC (3,92 ± 2,12) and the ovarian cyst group (1,10 ± 0,51). Based on Figure 1, it can be concluded that in the low-grade EOC group, the VEGF rs699947 genotype has a

significant effect on the relative mRNA expression of VEGF (*p*-value = 0,000). The AA genotype shows the highest relative mRNA expression at 5,19, while the relative mRNA expressions for the CA and CC genotypes are 2,86 and 2,09, respectively. The correlation between the VEGF rs699947 genotype and the relative mRNA expression of VEGF in the low-grade EOC group is classified as strong positive as evidenced by a Spearman correlation coefficient of 0,824, indicating a high level of correlation between genotype and expression levels.

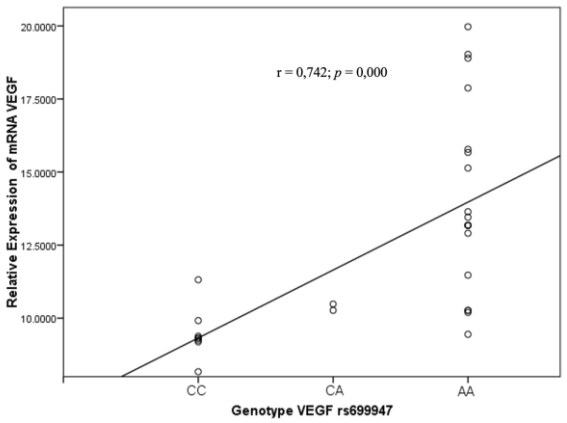

Figure 2. Correlation between the VEGF rs699947 genotype and VEGF mRNA expression levels in high-grade EOC

Based on Figure 2, it can be concluded that in the high-grade EOC group, the VEGF rs699947 genotype significantly affects the relative mRNA expression of VEGF (*p*-value = 0,000). The AA genotype demonstrates the highest relative mRNA expression, with a value of 14,03, while the relative mRNA expressions for the CC and CA genotypes are 9,48 and 10,37, respectively. The correlation between the VEGF rs699947 genotype and the relative mRNA expression of VEGF in the high-grade EOC group is classified as strong, as evidenced by a Spearman correlation coefficient of 0,742, indicating a strong positive correlation between the genotype and the levels of mRNA expression.

DISCUSSION

Ovarian cancer is infrequent in women below the age of 40, and the risk of developing ovarian cancer increases with age. In this study, the average age of women in the low-grade EOC group was 51.61 years, while the average age in the high-grade EOC group was 52.51 years. These findings are consistent with studies showing that the incidence of ovarian cancer increases in women after menopause.[17] Several factors may influence the development of ovarian cancer, including the use of Hormone Replacement Therapy (HRT) and environmental factors, such as exposure to carcinogenic agents.[18] Therefore, women over the age of 50 are at a higher risk of developing the disease.

Research on the correlation between the VEGF rs699947 genetic variant and ovarian cancer has been conducted across various populations. In this study, the AA genotype of VEGF rs699947 in the case group exhibited a higher frequency compared to reports from China and India. These findings align with previous studies conducted by Li et al. and Zhang et al. in China, which also identified a higher prevalence of the AA genotype in ovarian cancer patients.[16] However, contrasting results were reported in two earlier studies. One by Jia et al. demonstrated a higher frequency of the CA genotype in the case group compared to the control group.[16] Similarly, a study by Janardhan et al. in India reported a higher frequency of the CC genotype in the case group relative to the control group.[19]

These discrepancies in the research findings regarding the VEGF rs699947 genetic variant and ovarian cancer can be attributed to several factors.[20] Primarily, differences in ethnic backgrounds and genetic diversity across the studied populations may influence the distribution of genotypes. Furthermore, lifestyle and environmental factors, including exposure to carcinogens or other external agents, may interact with genetic predispositions in complex ways, contributing to variations in the risk of ovarian cancer across diverse populations.[20] These factors emphasize the importance of considering both genetic and environmental influences in the study of cancer risk, as they may interact to modulate the development of the disease.

The A allele of VEGF rs699947 is associated with a higher risk and potential for the development of EOC compared to the C allele, which appears to have a protective effect against EOC. The findings of this study are consistent with three previous studies conducted in China, which demonstrated that the frequency of the A allele of VEGF rs699947 was higher in the EOC case group compared to the control group. Conversely, the C allele was found at a lower frequency in the case group compared to the control group.[16] However, a different result was observed in a study from India, where the frequency of the C allele in VEGF rs699947 was higher in the EOC case group compared to the control group.[19] The discrepancy between the findings of this study and the previous studies conducted in India may be attributed to several factors, including differences in ethnic backgrounds within the populations studied, the sample sizes used, and variations in the clinicopathological characteristics of the ovarian cancer patients included in the research.

In this research, the genetic variation of the VEGF rs699947 polymorphism in both the control and case groups did not adhere to Hardy-Weinberg equilibrium (HWE). The Hardy-Weinberg law is a principle in genetics that states the allele frequencies in a population will remain stable across generations, provided no evolutionary factors affect the population.[21] The deviation from HWE observed in this study indicates that factors influencing the distribution of alleles and genotypes, such as gene mutation, natural selection, non-random mating, genetic drift, and gene flow, may be at play.[21] Gene mutation occurs when the DNA sequence changes, either creating new alleles or altering existing ones, which can affect allele frequencies in the population. Natural selection leads to changes in allele frequencies as individuals with more advantageous traits are more likely to survive and reproduce. Non-random mating happens when individuals select mates based on certain genetic factors, influencing allele and genotype

distribution. Genetic drift refers to random changes in allele frequencies, particularly in small populations, which can cause certain alleles to become more dominant or disappear. Gene flow involves the movement of individuals between different populations, introducing new alleles and altering the genetic structure of the population, which can result in deviations from HWE.[21]

VEGF is recognized as one of the key angiogenic factors involved in various physiological and pathological conditions. The evaluation of VEGF product expression in cancer tissue is crucial for understanding its role in tumor angiogenesis. In this study, the relative mRNA expression of VEGF was significantly higher in patients with high-grade epithelial ovarian cancer. These findings are consistent with previous studies conducted in India, which demonstrated that 42 out of 50 (84%) cases of epithelial ovarian cancer tested positive for both VEGF protein expression and mRNA expression, which were significantly higher in carcinomas compared to benign and borderline neoplasms.[22] The increased VEGF expression is attributed to a shift in the balance between pro-angiogenic and anti-angiogenic factors through a mechanism known as the "angiogenic switch." This refers to the transition of tumors from an avascular phase to a vascular phase, which then triggers hypervascularization in cancer cells.[23]

Various factors regulate the increased expression of VEGF, one of which is the hypoxic condition of the tumor microenvironment[5], along with cytokine influences via the PI3K/AKT pathway.[24] Hypoxia is a condition where the tumor microenvironment experiences oxygen deprivation, which is closely associated with the angiogenesis process that facilitates tumor growth.[25] HIF-1α (Hypoxia-Inducible Factor 1α) is degraded by the VHL (von Hippel-Lindau) protein under normoxic conditions, preventing it from dimerizing with HIF-1β and binding to the VEGF gene promoter. However, in cancer cells under hypoxic conditions, VHL is inactive, leading to the stabilization of HIF-1α. The stabilized HIF-1α then dimerizes with HIF-1β to form a heterodimer, HIF-1.[26] When cellular oxygen concentration decreases, HIF-1 activates a number of genes that contain hypoxia response elements (HRE) on their promoters, including VEGF, to enhance oxygen supply as a response to hypoxia. This HIF-1 dimer binds to the VEGF gene promoter, resulting in increased VEGF expression and further driving the process of tumor angiogenesis.[26]

The increased expression of VEGF can also be triggered by cancer cell proliferation induced by hypoxia.[27] In the tumor microenvironment, uncontrolled cancer cell proliferation may surpass the capacity of existing blood vessels to meet the oxygen demands of the tumor cells, which typically occurs when the tumor diameter exceeds 1 mm.[28] In response to decreased oxygen levels, tumor cells adapt by increasing VEGF expression to promote the formation of new blood vessels.[28] Additionally, cancer cell proliferation can be facilitated by survivin, a protein involved in cancer progression by enhancing angiogenesis. The expression of survivin is regulated by the activity of the β-catenin/Tcf-Lef transcription factor through a signaling mechanism dependent on the PI3K/AKT pathway, which in turn upregulates VEGF gene expression.[24] Increased VEGF expression can also be influenced by the interaction between CD40 and its ligand, which is elevated in inflammatory conditions and is believed to enhance tumor growth and development through the activation of the RAS signaling pathway.[29] RAS activation, in turn, links to the PI3K/AKT pathway, inducing VEGF activity as a key mediator in the process of new blood vessels formation within the tumor microenvironment.[29]

The highest mRNA VEGF expression was found in high-grade epithelial ovarian cancer, primarily due to the fact that the majority of the samples in this group were from high-grade serous carcinoma, with a total of 27 samples. High-grade serous carcinoma is often associated with mutations in the TP53 gene.[30] Several previous studies on multiple myeloma, pancreatic cancer, and renal cancer have shown that TP53 mutations can increase VEGF expression through angiogenesis induced by CD40 ligation.[31] Further research also explains that TP53 mutations play a crucial role in enhancing VEGF expression, as mutant TP53 collaborates with the subunit of the chromatin remodeling complex Switch/Sucrose Non-Fermentable (SWI/SNF).[32] This complex functions to alter nucleosome and DNA structures during transcription. In this way, mutant TP53 can bind to the transcription initiation sites on the VEGF gene promoter through the activation of the "angiogenic switch," ultimately leading to an increase in VEGF expression.[32]

In this study, a strong positive correlation and significant relationship were found between the VEGF rs699947 genotype and the relative mRNA VEGF expression levels in both low-grade and high-grade EOC groups. Additionally, the VEGF rs699947 AA genotype plays a role in the increased mRNA VEGF expression in ovarian cancer patients. To date, no other studies have reported a correlation between the VEGF rs699947 genotype and VEGF mRNA expression in ovarian cancer. Two previous studies conducted on colorectal cancer patients in Australia and Japan showed similar results, where the VEGF rs699947 AA genotype was associated with increased VEGF mRNA expression in colorectal cancer patients.[33] This is because VEGF rs699947 is located in the promoter region of the VEGF gene, which can influence the promoter's activity in binding specific transcription factors, thus controlling the initiation of transcription and inducing a quantitative increase in VEGF expression in ovarian cancer.[34]

CONCLUSION

In this study, significant differences were observed in the distribution of the VEGF rs699947 genotype and allele between the normal group and the EOC case groups. The relative VEGF mRNA expression levels were significantly higher in both the low-grade EOC and high-grade EOC groups compared to the ovarian cyst group. Moreover, a significant difference in relative mRNA expression was observed between the low-grade and high-grade EOC case groups. The elevated VEGF mRNA expression in EOC cases was associated with the AA genotype of rs699947, which was reflected by a higher frequency of the A allele in the case groups. This genotype was linked to an increased risk of EOC, with an

odds ratio of 4.439, indicating a 4.439-fold higher likelihood of developing EOC. In contrast, individuals possessing the CC genotype exhibited the lowest levels of VEGF mRNA expression in both the low-grade and high-grade EOC case groups. These findings are further supported by the odds ratio of 0.225 for the C allele, which suggests a protective effect against the development of EOC.

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REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021;149(4):778–89. doi: https://doi.org/10.1002/ijc.33588
2. Instalasi Pengendali Data Beban Kanker dan Jejaring Kanker Nasional. Profil Kanker RS. Kanker Dharmais. Kemenkes RSK Dharmais. 2020. p. 25.
3. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA Cancer J Clin. 2019 Jul 17;69(4):280–304. doi/10.3322/caac.21559
4. González-Martín A, Harter P, Leary A, Lorusso D, Miller RE, Pothuri B, et al. Newly diagnosed and relapsed epithelial ovarian cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2023 Oct;34(10):833–48. doi: https://doi.org/10.1016/j.annonc.2023.07.011
5. Lorenc P, Sikorska A, Molenda S, Guzniczak N, Dams-Kozlowska H, Florczak A. Physiological and tumor-associated angiogenesis: Key factors and therapy targeting VEGF/VEGFR pathway. Biomed Pharmacother [Internet]. 2024 Nov;180:117585. doi: https://doi.org/10.1016/j.biopha.2024.117585
6. Saman H, Raza SS, Uddin S, Rasul K. Inducing Angiogenesis, a Key Step in Cancer Vascularization, and Treatment Approaches. Cancers (Basel). 2020 May 6;12(5):1172. doi: https://doi.org/10.3390/cancers12051172
7. Pérez-Gutiérrez L, Ferrara N. Biology and therapeutic targeting of vascular endothelial growth factor A. Nat Rev Mol Cell Biol. 2023 Nov 25;24(11):816–34. doi: https://doi.org/10.1038/s41580-023-00631-w
8. Ye X, Gaucher JF, Vidal M, Broussy S. A Structural Overview of Vascular Endothelial Growth Factors Pharmacological Ligands: From Macromolecules to Designed Peptidomimetics. Molecules . 2021 Nov 9;26(22):6759. doi: https://doi.org/10.3390/molecules26226759
9. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, et al. Role of Matrix Metalloproteinases in Angiogenesis and Cancer. Front Oncol. 2019 Dec 6;9. doi: https://doi.org/10.3389/fonc.2019.01370
10. Ullah I, Abu-Dawud R, Busch JF, Rabien A, Erguen B, Fischer I, et al. VEGF – Supplemented extracellular matrix is sufficient to induce endothelial differentiation of human iPSC. Biomaterials . 2019 Sep;216:119283. doi: https://doi.org/10.1016/j.biomaterials.2019.119283
11. Wang G, Heij LR, Liu D, Dahl E, LANG SA, Ulmer TF, et al. The Role of Single-Nucleotide Polymorphisms in Cholangiocarcinoma: A Systematic Review. Cancers (Basel). 2022 Dec 2;14(23):5969. doi: https://doi.org/10.3390/cancers14235969
12. Wu YL, Lin ZJ, Li CC, Lin X, Shan SK, Guo B, et al. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. Signal Transduct Target Ther. 2023 Mar 2;8(1):98. doi: https://doi.org/10.1038/s41392-023-01333-7
13. Suvanto M, Beesley J, Blomqvist C, Chenevix-Trench G, Khan S, Nevanlinna H. SNPs in lncRNA Regions and Breast Cancer Risk. Front Genet . 2020 Jun 30;11. doi: https://doi.org/10.3389/fgene.2020.00550
14. Bhaskari J, Premalata CS, Shilpa V, Rahul B, Pallavi VR, Ramesh G, et al. Vascular endothelial growth factor polymorphisms and a synchronized examination of plasma and tissue expression in epithelial ovarian cancers. Tumor Biol [Internet]. 2016 Jan 13;37(1):1017–23. doi: https://doi.org/10.1007/s13277-015-3891-3
15. Al-Mohaya MA, Alfadhel AK, Mustafa M, Alquwayz TS, Al-Anazi MA. Vascular endothelial growth factor (VEGF-2578 C > A) gene polymorphism as a genetic biomarker for breast cancer: A case control study. Gene Reports. 2021 Mar;22:101007. doi: https://doi.org/10.1016/j.genrep.2020.101007
16. Xu CH, He ZH, Xu H. Association of four genetic polymorphisms in the vascular endothelial growth factor-A gene and development of ovarian cancer: a meta-analysis. Oncotarget. 2017 Sep 22;8(42):73063–78. doi: https://doi.org/10.18632/oncotarget.20379
17. Care NI for H and, Excellence. Menopause (update) Ovarian Cancer. Vol. 3. National Library of Medicine, NCBI; 2024.
18. Liu Y, Ma L, Yang X, Bie J, Li D, Sun C, et al. Menopausal Hormone Replacement Therapy and the Risk of Ovarian Cancer: A Meta-Analysis. Front Endocrinol (Lausanne) [Internet]. 2019 Dec 3;10. doi: https://doi.org/10.3389/fendo.2019.00801
19. Janardhan B, Vaderhobli S, Bhagat R, Chennagiri Srinivasamurthy P, Venketeshiah Reddihalli P, Gawari R, et al. Investigating impact of Vascular Endothelial Growth Factor Polymorphisms in Epithelial Ovarian Cancers: A Study in the Indian Population. Tang CH, editor. PLoS One [Internet]. 2015 Jul 9;10(7):e0131190. doi: 10.1371/journal.pone.0131190

20. Herrera-Luis E, Benke K, Volk H, Ladd-Acosta C, Wojcik GL. Gene–environment interactions in human health. Nat Rev Genet [Internet]. 2024 Nov 28;25(11):768–84.doi: https://doi.org/10.1038/s41576-024-00731-z
21. Gupta P. Population Genetics. In: Genetics Fundamentals Notes [Internet]. Singapore: Springer Nature Singapore; 2022. p. 1077–103. doi: https://doi.org/10.1007/978-981-16-7041-1_21
22. Mukherjee S. VEGF Expression to Support Targeted Therapy in Ovarian Surface Epithelial Neoplasms. J Clin Diagnostic Res. 2017; doi: 10.7860/JCDR/2017/24670.9737
23. Geindreau M, Ghiringhelli F, Bruchard M. Vascular Endothelial Growth Factor, a Key Modulator of the Anti-Tumor Immune Response. Int J Mol Sci [Internet]. 2021 May 4;22(9):4871. doi: https://doi.org/10.3390/ijms22094871
24. He Y, Sun MM, Zhang GG, Yang J, Chen KS, Xu WW, et al. Targeting PI3K/Akt signal transduction for cancer therapy. Signal Transduct Target Ther [Internet]. 2021 Dec 16;6(1):425. doi: https://doi.org/10.1038/s41392-021-00828-5
25. Chen Z, Han F, Du Y, Shi H, Zhou W. Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. Signal Transduct Target Ther [Internet]. 2023 Feb 17;8(1):70. doi: https://doi.org/10.1038/s41392-023-01332-8
26. Emami Nejad A, Najafgholian S, Rostami A, Sistani A, Shojaeifar S, Esparvarinha M, et al. The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. Cancer Cell Int. 2021 Jan 20;21(1):62. doi: https://doi.org/10.1186/s12935-020-01719-5
27. Patel SA, Nilsson MB, Le X, Cascone T, Jain RK, Heymach J V. Molecular Mechanisms and Future Implications of VEGF/VEGFR in Cancer Therapy. Clin Cancer Res. 2023 Jan 4;29(1):30–9. doi: https://doi.org/10.1158/1078-0432.CCR-22-1366
28. de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell. 2023 Mar;41(3):374–403. doi: https://doi.org/10.1016/j.ccell.2023.02.016
29. Shaw P, Dwivedi SKD, Bhattacharya R, Mukherjee P, Rao G. VEGF signaling: Role in angiogenesis and beyond. Biochim Biophys Acta - Rev Cancer . 2024 Mar;1879(2):189079. doi: https://doi.org/10.1016/j.bbcan.2024.189079
30. Santoro A, Angelico G, Travaglino A, Inzani F, Spadola S, Pettinato A, et al. The multiple facets of ovarian high grade serous carcinoma: A review on morphological, immunohistochemical and molecular features. Crit Rev Oncol Hematol. 2025 Apr;208:104603. doi: https://doi.org/10.1016/j.critrevonc.2024.104603
31. Li AM, Boichard A, Kurzrock R. Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues. Cancer Biol Ther [Internet]. 2020 Jan 2;21(1):95–100. doi: https://doi.org/10.1080/15384047.2019.1665956
32. Ruzzo A, Graziano F, Palladino S, Fischer NW, Catalano V, Giordani P, et al. Clinical impact of TP53 functional mutations in patients with metastatic colorectal cancer treated with bevacizumab and chemotherapy. Oncologist. 2024 Oct 22; doi: https://doi.org/10.1093/oncolo/oyae277
33. Chionh F, Gebski V, Al-Obaidi SJ, Mooi JK, Bruhn MA, Lee CK, et al. VEGF-A, VEGFR1 and VEGFR2 single nucleotide polymorphisms and outcomes from the AGITG MAX trial of capecitabine, bevacizumab and mitomycin C in metastatic colorectal cancer. Sci Rep. 2022 Jan 24;12(1):1238 doi: https://doi.org/10.1038/s41598-021-03952-y
34. Palmer BR, Paterson MA, Frampton CM, Pilbrow AP, Skelton L, Pemberton CJ, et al. Vascular endothelial growth factor-A promoter polymorphisms, circulating VEGF-A and survival in acute coronary syndromes. Zirlik A, editor. PLoS One. 2021 Jul 14;16(7):e0254206. doi: https://doi.org/10.1371/journal.pone.0254206