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Original Article

World J Oncol. 2025;16(3):295-298

Assessing the Impact of *VEGF* -2578C>A (rs699947) and -7C>T (rs2010963) Polymorphisms on Bladder Cancer Susceptibility: A Pilot Study

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Abstract

Background: One of the most common cancers is bladder cancer, characterized by a high mortality rate and a high incidence of recurrence. An identified susceptibility factor for bladder cancer is the presence of single nucleotide polymorphisms (SNPs) in vascular endothelial growth factor (*VEGF*) genes, specifically -2578C>A and -7C>T polymorphisms.

Methods: This pilot study was conducted to uncover the association between the SNP *VEGF* -2578C>A and -7C>T in bladder cancer. A total of 20 patients were tested, consisting of both 10 controls and patients with bladder cancer, for the SNP of *VEGF* -2578C>A and -7C>T through polymerase chain reaction (PCR).

Results: This study showed a significant association between the *VEGF* -2578C>A polymorphism (odds ratio (OR) 1.93, 95% confidence interval (CI) 1.03 - 2.01) and an increased risk of bladder cancer, especially in individuals with the A allele. However, the results for the -7C>T polymorphism in relation to bladder cancer risk showed a non-significant association in this study population. We did not detect a significant association between the *VEGF* -2578C>A (OR 0.93 (0.39 - 2.01)) and -7C>T (OR 0.74 (0.32 - 1.57)) polymorphisms and bladder cancer severity, as indicated by stages.

Conclusion: This pilot study suggests that *VEGF* -2578C>A has a connection between polymorphism and bladder cancer susceptibility. Further large-scale studies need to be done to validate these findings and elucidate the underlying mechanisms linking *VEGF* polymorphisms to bladder cancer pathogenesis.

Keywords: Single nucleotide polymorphism; *VEGF*; Bladder cancer; Risk factor

Manuscript submitted March 17, 2025, accepted May 27, 2025
Published online June 14, 2025

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doi: https://doi.org/10.14740/wjon2515

Introduction

Bladder cancer is a malignancy that occurs frequently both globally and specifically in Indonesia. Bladder cancer's incidence bears a major health burden. By definition, bladder cancer involves the invasion of neoplastic cells from the urothelial into the lamina propria or deeper layers. The risk factors associated with bladder cancer are multifactorial, encompassing genetics, environment, and lifestyle. Important risk factors influencing bladder cancer development are smoking, which increases the risk by 2 - 6 times compared to non-smokers, schistosomiasis infection, which causes irritation and chronic inflammation leading to metaplasia, and occupational exposure to petroleum products and chemicals [1].

In recent years, the contribution of genetic factors to the increased risk of bladder cancer has become more widely recognized. Numerous genetic loci linked to this increased risk have been found by genome-wide association studies (GWAS). Specifically, pathologically relevant single nucleotide polymorphisms (SNPs) have been found in genes linked to angiogenesis, cell proliferation, and death [2].

Angiogenesis is a basic mechanism underlying cancer development, invasion, and metastases. Regarding cancer, the angiogenesis process is dysregulated, with an imbalance between pro-angiogenic and antiangiogenic factors, resulting in pathological conditions. Vascular endothelial growth factor (*VEGF*) is a key factor in the angiogenesis process that facilitates endothelial cell proliferation, migration, and vascular permeability [2].

Numerous *VEGF* isoforms are produced by the *VEGF* gene, which is located on chromosome 6p21.3. These isoforms bind to endothelial cell *VEGF* receptors (VEGFRs) to stimulate angiogenesis. Genetic polymorphisms within the *VEGF* gene, particularly in regulatory regions that govern gene expression and protein synthesis, have been linked to progno-

sis and susceptibility to cancer. The *VEGF* polymorphisms -2578C>A (rs699947) and -7C>T (rs2010963) are commonly examined. They have been linked to variations in *VEGF* expression levels and have been investigated in bladder cancer among other tumors [2, 3].

Numerous studies have linked *VEGF* polymorphisms to bladder cancer risk; however, most of them were carried out in homogeneous populations with little representation of different ethnic groups. The differences in results from various studies can be attributed to several factors, including variations in study design, such as sample size, ethnicity, genotyping methods, and environmental exposures. Additionally, the complex interactions of genetic factors with other risk factors, such as smoking, occupational exposure, and lifestyle, require further analysis to account for confounding variables. Therefore, additional research is required to define the correlation between *VEGF* polymorphisms in bladder cancer risk [2, 3].

Materials and Methods

Designing the pilot study

To elucidate the link between bladder cancer risk and the *VEGF* -2578C>A polymorphisms, this pilot study was designed as a case-control analysis. The study intended to offer a framework for next more extensive studies by first offering basic understanding of the correlation between bladder cancer and *VEGF* polymorphisms.

Planning and preparation of data collection

Institutional Review Board (IRB) approval was obtained to guarantee the research was carried out in compliance with moral standards and patient privacy. A detailed protocol covering the study objectives, methodology, inclusion criteria, and data collection procedures was developed.

Genotyping analysis by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to validate the *VEGF* -2578C>A and -7C>T polymorphisms. Quality control measures were implemented to ensure the genotyping results' repeatability and accuracy.

Participants

This study included a total of 20 participants, separated into two groups of 10: a control group and a case group positive for the *VEGF* -2578C>A and -7C>T polymorphisms. The control group consisted of individuals who had never been diagnosed with bladder cancer or other cancers, matched based on age, sex, and ethnicity. The case group comprised individuals diagnosed with bladder cancer who were undergoing therapy at the institution where the study was conducted.

Inclusion criteria for individuals in the case group included those with diagnosed and histologically confirmed bladder cancer, as well as the availability of DNA samples for genotyping analysis. Exclusion criteria comprised a history of any previous cancer, prior chemotherapy or radiotherapy for bladder cancer, and individuals who did not provide informed consent.

Data collection

Patient data were collected through interviews and medical records. Demographic and clinical data included age, sex, smoking status, tumor stage, and grade. Peripheral blood samples were acquired from study participants for DNA extraction using standardized protocols.

PCR-RFLP analysis was performed to validate the genotypes of *VEGF* -2578C>A and -7C>T polymorphisms. The analysis involved amplifying genomic DNA using allele-specific primers that target the polymorphic regions of the *VEGF* gene. Subsequently, to identify genotypes, the PCR results were digested using restriction enzymes and examined on an agarose gel.

Data analysis and findings

Descriptive statistics were used to examine the clinical and demographic information of the study subjects. Bladder cancer sensitivity was found by means of the Chi-square test, sometimes known as Fisher's exact test, between the *VEGF* -2578C>A and -7C>T polymorphisms. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure the strength of the association.

According to preliminary results, *VEGF* -2578C>A polymorphism clearly correlates with a higher bladder cancer risk. Individuals with the A allele exhibit a higher risk of bladder cancer compared to those with the CC genotype. However, in the study group, no significant association was found between the -7C>T polymorphism and bladder cancer risk.

The results of these preliminary findings serve as the basis for conducting larger-scale studies to better understand how bladder cancer etiology interacts with *VEGF* polymorphisms. Additionally, these preliminary results provide an insight into bladder cancer susceptibility and its association with *VEGF* polymorphisms.

Results

A total of 20 patients participated in the study, consisting of 10 control patients and 10 patients positive for *VEGF* polymorphisms -2578C>A and -7C>T. The median age was 72 (range 54 - 84) years, and the majority of participants were male (70%), with 60% identifying as Batak ethnicity. This study indicated that 88% of subjects were heavy smokers, and 80% of subjects had a family history of cancer. Regarding bladder cancer susceptibility, *VEGF* -2587C>A ($P < 0.05$) showed a statistically significant connection; this was not true of the -7C>T polymorphism. Our study indicated a relatively high risk of bladder cancer in subjects with *VEGF* -2587C>A polymorphism (OR 1.93, 95% CI 1.03 - 2.01). However, based

on the stages, we found no appreciable correlation between bladder cancer severity and *VEGF* -2578C>A (OR 0.93 (0.39 - 2.01)) and -7C>T (OR 0.74 (0.32 - 1.57)).

Discussion

Among urinary tract cancers, bladder cancer is the most often occurring one. In the United States, bladder malignancy affects males roughly 45,000 times and women roughly 17,000 times. In Indonesia, bladder cancer ranks among the top 10 malignancies in men, and its incidence has increased over the last decade [1]. Globally, the incidence of bladder cancer reaches 9.6 per 10,000 for men and 2.4 per 100,000 for women. Age is a significant risk factor for bladder cancer, which is typically diagnosed in middle-aged and older adults, with a median age of around 70 years. Consistent with our findings, several demographic studies have shown that individuals over 65 years have an 11-fold higher risk of developing cancer [1, 2].

Environmental and genetic aspects both affected bladder cancer susceptibility. Another factor associated with bladder cancer is smoking, which is linked to carcinogenic substances in cigarettes, including o-toluidine and 4-aminobiphenyl. Smokers have a fourfold higher risk compared to non-smokers, including both active and passive smokers [3]. Additionally, cigarette smoking has been found to increase cell proliferation, characterized by urinary tract epithelial hyperplasia [4]. Our study also indicated that 88% of subjects were heavy smokers. According to present studies, smoking was positively linked to bladder cancer risk since the rate of smoking in bladder cancer cases was higher than that in controls. However, bladder cancer is not always attributed to exposure to these risk factors, suggesting that genetic susceptibility may also play a significant role in the disease [5, 6]. Bladder cancer patients are known to exhibit an imbalance between oncogenes and tumor suppressor genes, as well as increased activity of factors involved in angiogenesis (e.g., *VEGF*) [6, 7]. This hereditary susceptibility can manifest as various malignancies inherited by subsequent generations. Our research revealed that a family history of cancer is a statistically significant risk factor.

In this study, we found a relatively high risk of bladder cancer in subjects with *VEGF* -2587C>A polymorphism. Located on chromosome 6p21.3, the *VEGF* gene comprised eight exons with alternative splicing to generate a family of proteins [8]. Different kinds of cancer, including oral, breast, glioma, colorectal, and lung, have been linked in certain studies to SNPs within *VEGF* [9]. *VEGF* is pivotal to the neovascular growth required to sustain solid tumor progression [10]. Although several studies have looked at the link between *VEGF* polymorphisms and bladder cancer risk, their findings have been conflicting. The study by Zhu et al demonstrated a significant association between *VEGF* polymorphisms and an increased risk of bladder cancer in the Asian population, particularly with the -2578C>A polymorphism [5]. However, subgroup analyses revealed heterogeneity across the studies, indicating that further research is needed. Both the studies by Lee et al and Wang et al found a significant association between -7C>T polymorphism and an increased risk of bladder cancer in the Korean and Chinese populations, respectively [1, 2]. However, these results have not been consistently replicated in other populations, with multiple investigations failing to show a substantial association between the -7C>T polymorphism and the incidence of bladder cancer.

The involvement of various genetic variations in clinicopathological processes and susceptibility to bladder cancer has been reported in multiple studies. Kouidhi et al synthesized data from 27 case-control studies involving 15,013 participants and found that the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase gene were associated with a higher incidence of bladder cancer and poorer outcomes [11]. Another study by Li et al examined six case-control studies with 1,328 participants and identified an association between bladder cancer and polymorphisms in *p53* codon 72, suggesting that the distribution of these variants may vary depending on disease stage [12]. Additionally, a meta-analysis by Jiang et al included 10 case-controls with 3,549 participants and found that the *TP53* Arg72Pro genotype is highly susceptible to bladder cancer in Asian populations [13]. Kellen et al proposed a possible association between the glutathione S-transferase P1 Ile105Val polymorphism and a slightly elevated risk of bladder cancer in their HuGE-GSEC review [14]. Increased *VEGF* levels influenced by *VEGF* genetic polymorphisms are associated with cancer susceptibility and affect disease prognosis.

Individuals with the AA genotype and the A allele have a higher risk of developing bladder cancer compared to those with the AC and CC genotypes and the C allele. However, this polymorphism shows no significant association with the stage and grade of bladder cancer. Referring to the previous discussion that bladder cancer progression is regulated by blood *VEGF* levels, and that serum *VEGF* levels themselves are associated with the *VEGF* -2578C>A polymorphism, it can be concluded that this polymorphism indirectly affects bladder cancer progression. The relationship between the two is mediated by serum *VEGF* levels.

Our study has various restrictions. First of all, this study had a somewhat tiny sample size. Second, the future research should incorporate more SNPs found in *VEGF* gene. Thirdly, we did not assess the interactions of these SNPs with the plasma *VEGF* levels, which might possibly indicate patients' illness condition.

Conclusion

This pilot study provides preliminary evidence suggesting a potential role and association between the *VEGF* -2578C>A polymorphism and bladder cancer susceptibility. Our study also indicated that the rate of smoking was higher in bladder cancer cases than that in controls, indicating that smoking was positively associated with bladder cancer risk. This pilot study is limited by its small sample size, which may affect the generalizability of the findings. Larger, multi-center studies are warranted to validate these preliminary findings and elucidate the underlying mechanisms linking *VEGF* polymorphisms to bladder cancer pathogenesis.

Acknowledgments

The authors would like to express their sincere gratitude to all individuals and institutions who contributed to the completion of this study.

Financial Disclosure

None to declare.

Conflict of Interest

The authors report no conflict of interest.

Informed Consent

Not applicable.

Author Contributions

Concept, methodology, data curation, formal analysis, investigation, project administration, software, writing original draft, and writing review editing: GPS and IP. Funding, resources, supervision, and writing review editing: GPS and FS. Validation, visualization, resources, supervision, and writing review editing: FS and TT.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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