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Vitamin D Receptor Polymorphisms and Nasopharyngeal Carcinoma Risk in the Minangkabau Ethnic Group

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Abstract

Nasopharyngeal carcinoma (NPC) is a multifactorial malignancy influenced by genetic, environmental, and viral factors. The Vitamin D Receptor (VDR) gene has been of particular interest due to its role in cellular processes relevant to carcinogenesis. This study investigated the potential association of VDR gene BsmI (rs1544410) and FokI (rs2228570) polymorphisms and NPC. Methods. A total of 36 cases of NPC and 23 controls participated in the study. All patients and controls in this study were from the Minangkabau ethnic group in the West Sumatra region of Indonesia. Genomic DNA was extracted from whole blood using a DNA purification kit. DNA fragments were amplified by PCR. The VDR variants BsmI and FokI were genotyped by PCR sequencing. Results. The frequencies of the bb and Bb carrier genotypes of VDR BsmI were 86.1 and 13.9 % in NPCs and 73.9 and 26.1% in the controls. The frequencies of the ff, Ff, and FF genotypes of VDR FokI were 11.1, 47.2, and 41.7% in NPCs and 8.7, 69.6, and 21.7% in the controls. The frequencies of b and B alleles of VDR BsmI were 93.1 and 6.9% in cases and 87.0 and 13.0% in the controls. The frequencies of f and F alleles of VDR Fok I were 34.7 and 65.3% in cases and 43.5 and 56.5% in the controls. No significant differences were observed in the genotype distributions and allele frequencies of the VDR BsmI and FokI polymorphisms between the cases and controls ($p > 0.05$). Our study reveals no association between VDR gene BsmI and FokI polymorphisms and NPC in the Minangkabau ethnic group.

Keywords: Times VDR gene polymorphisms, BsmI, FokI, Nasopharyngeal Carcinoma, Minangkabau

Full length article **Corresponding Author*, e-mail: sukrirahman@med.unand.ac.id

1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor arising from the epithelial lining of the nasopharynx. It is known to have a multifactorial etiology involving genetic, environmental, and viral factors [1]. Genetic variations play a pivotal role in modulating an individual's susceptibility to NPC [2]. Among the genetic factors, the Vitamin D receptor (VDR) gene has attracted significant attention due to its involvement in various cellular processes, including cell growth regulation, immune response, and apoptosis. Several studies have been done to investigate the association between the serum level of vitamin D and the risk of various cancers [3]. A study by Pu et al. reported that elevated levels of vitamin D by diet intake, genomic polymorphisms, and circulated 25-OHD may protect people from head and neck cancer [4]. Polymorphisms in the VDR gene, such as bsmI and fokI, have been investigated for their potential association with various cancers [5,6,7]. The bsmI polymorphism involves a restriction site in the 3' untranslated region of the VDR gene, while the fokI polymorphism occurs in the start codon region, influencing the VDR protein's structure and function. These polymorphisms have been linked to altered VDR activity, potentially affecting vitamin D metabolism and signaling pathways.

Given the emerging role of vitamin D in immune regulation, inflammation, and cell differentiation, investigating the relationship between VDR gene polymorphisms and NPC risk is paramount. Understanding how these genetic variations influence an individual's susceptibility to NPC could offer insights into the underlying mechanisms of carcinogenesis and aid in identifying high-risk populations. This study aims to explore the potential association between VDR gene bsmI and fokI polymorphisms and the risk of nasopharyngeal carcinoma in the Minangkabau ethnic group. By elucidating the genetic

factors contributing to NPC susceptibility, we hope to contribute to a deeper understanding of the disease's pathogenesis and potentially pave the way for personalized preventive and therapeutic strategies.

2. Materials and methods

This study was carried out on the Minangkabau ethnic group. The study was approved by the Faculty of Medicine Andalas University ethics committee, Padang Indonesia (No.569/UN.16.2/KEP-FK/2022), and written informed consent was obtained from participants in both NPC and control groups. All patients and controls in this study were from the Minangkabau ethnic group in the West Sumatra region of Indonesia. The study included 36 NPC patients diagnosed in Dr. M. Djamil General Hospital. At the time of diagnosis, the patients were between 20 and 78 years of age, with a median age of 48. The controls were healthy people who visited the hospital. A total of 23 controls participated in the study. A 10 ml whole blood sample was taken from each study subject for DNA extraction. Genomic DNA was extracted from whole blood using a DNA purification kit. DNA fragments were amplified by PCR. The primers used for amplification are listed in Table 1. The VDR variants BsmI and FokI were genotyped by PCR sequencing at 1stBASE, Malaysia. The software Geneious 11.1.2 was used to determine the genotype. The Chi-square test was used to assess any association between VDR polymorphisms and NPC risk, and 95% confidence intervals (CIs) were calculated to determine the risk of NPC associated with a given VDR genotype.

3. Results

A total of 36 NPC patients and 23 healthy controls were recruited in this study. Table 2 shows the characteristics of the participants. The case group (22 males and 14 females) had a mean age ± SD of 48.5 ±13.11 years. The control group consisted of 23 healthy volunteers who visited the M. Djamil General Hospital at Padang, West Sumatra, Indonesia. The mean age ±S.D. of the control group (14 males and nine females) was 45.8 ±13.62 years. Table 3 shows the distribution of VDR gene polymorphism genotypes in the NPC and control groups. The frequencies of the bb and Bb carrier genotypes of VDR BsmI were 86.1 and 13.9 % in NPCs and 73.9 and 26.1% in the controls. The frequencies of the ff, Ff, and FF genotypes of VDR FokI were 11.1, 47.2, and 41.7% in NPCs and 8.7, 69.6, and 21.7% in the controls. Statistically, there were no significant differences between NPC and control groups in the genotype distributions ($p>0.05$). Table 4 shows the proportion of VDR gene polymorphism alleles in the NPC and control groups. The frequencies of b and B alleles of VDR BsmI were 93.1 and 6.9% in the cases group and 87.0 and 13.0% in the controls. The frequencies of f and F alleles of VDR Fok I were 34.7 and 65.3% in cases and 43.5 and 56.5% in the controls. No significant differences were observed in the allele frequencies of the VDR Bsm I and FokI polymorphisms between the cases and controls ($p> 0.05$).

4. Discussion

The etiology of nasopharyngeal carcinoma is still not fully understood. It is believed to be multifactorial, with a complex interaction of Epstein-Barr virus infection, environmental factors, and genetic susceptibility playing a role. In recent years, there have been increasing studies investigating the potential relationship between vitamin D receptor gene polymorphisms and the risk of various cancer types, including nasopharyngeal carcinoma. The host immune system plays an important role in monitoring and controlling the spread of infections, including EBV [8]. Genetic polymorphisms in immune-reactive genes have been implicated in making a person more susceptible or resistant to infections. Genetic studies have shown that the association between HLA and non-HLA genes is responsible for infection susceptibility.

The vitamin D receptor is a member of the nuclear receptor family that controls transcriptional responses and regulates microRNA-directed post-transcriptional mechanisms to initiate effective immune responses; thus, vitamin D facilitates several immunomodulatory properties through VDR [9]. VDR is found in many immune cells, such as macrophages, dendritic cells, and T and B lymphocytes, and upon stimulation, VDR plays a dynamic role in the host immune response. The precise mechanisms by which vitamin D receptor polymorphisms contribute to nasopharyngeal carcinoma development are not fully understood. Studies have shown that polymorphisms in the vitamin D receptor gene, particularly the FokI and BsmI polymorphisms, may play a role in the development and progression of several cancers [3].

The vitamin D receptor (VDR) upregulates and downregulates many genes by binding to the vitamin D responsive element (VDRE), affecting several biological activities, such as calcium metabolism, immunity, detoxification, oxidative stress, cell proliferation, and differentiation. Many studies have shown that vitamin D reduces the risk of various malignancies. Recent studies and meta-analyses have evaluated the role of VDR polymorphisms [10]. Nevertheless, the role of VDR polymorphisms requires further investigation. Investigating the potential association between Vitamin D Receptor (VDR) gene bsmI and fokI polymorphisms and the risk of nasopharyngeal carcinoma (NPC) has provided valuable insights into the complex interplay between genetic factors and cancer susceptibility. Despite the initial hypothesis suggesting a potential link between these polymorphisms and NPC risk, our study did not identify a statistically significant association between VDR bsmI and fokI polymorphisms and the incidence of NPC. These findings shed light on the nuanced nature of genetic influences in the NPC context and suggest additional contributing factors. The result is consistent with the previous study by Huang et al. [11]. In contrast, Wang et al. [12] reported that the distribution of the FokI Ff genotype in the case group was significantly higher than that in the healthy control group. Our study found the frequencies of the ff, Ff, and FF genotypes of VDR FokI were 11.1, 47.2, and 41.7% in NPCs and 8.7, 69.6, and 21.7% in the controls.

Table 1. Primer of VDR gene polymorphisms

DVR SNPs	Amplicon (bp)	Primers for PCR Amplifications (5'-3')
BsmI A/G (B/b)	706	F: GGGAGTATGAAGGACAAAGACC
		R: CTGTGTCCCATTTGCTGCTG
FokI C/T (F/f)	717	F: ATGCATATGATCCTTACACCCTGG
		R: GCGATTTCCAAGAGAGTCAGAGG

SNP single-nucleotide polymorphism, BP base pairs, F forward, R reverse

Table 2. Characteristics of the study population

Variables	NPC f (%)	Control f (%)
Sex		
Male	22 (61.1)	15 (65.2)
Female	14 (38.9)	8 (34.8)
Age (years), Mean±SD	48.5±13.11	45.8±13.62
Clinical Stage		
I	2 (5.6)	
II	3 (8.3)	
III	4 (11.1)	
IV	27 (75.0)	
Histologic type		
Keratinized Carcinoma	0 (0.0)	
Undiff. non-keratinizing carcinoma	10 (27.8)	
Undifferentiated carcinoma (WHO III)	26 (72.2)	

Table 3. Genotype distributions of VDR polymorphisms

VDR SNPs		Group		p
		NPC f (%)	Control f (%)	
BsmI B/b (A/G)				
	bb (GG)	31 (86.1)	17 (73.9)	0.406
	Bb (GA)	5 (13.9)	6 (26.1)	
	BB (AA)	0 (0)	0 (0)	
FokI F/f (C/T)				
	ff (TT)	4 (11.1)	2 (8.7)	0.236
	Ff (CT)	17 (47.2)	16 (69.6)	
	FF (CC)	15 (41.7)	5 (21.7)	

Table 4. Allele frequencies of VDR polymorphisms

Allele		Group		p
		NPC f (%)	Control f (%)	
BsmI				
	b (G)	67 (93.1)	40 (87.0)	0.431
	B (A)	5 (6.9)	6 (13.0)	
FokI				
	f (T)	25 (34.7)	20 (43.5)	0.447
	F (C)	47(65.3)	26 (56.5)	

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Statistically, there were no significant differences between NPC and control groups in the genotype distributions (p>0.05). The role of VDR gene polymorphism in nasopharyngeal carcinoma might differ from its impact on other malignancies. The lack of a significant association suggests that other genetic, environmental, or lifestyle factors might substantially influence an individual's susceptibility to NPC. Moreover, the genetic landscape of NPC could be affected by population-specific variations, underscoring the need for further studies across diverse ethnic groups. The polymorphic form of the VDR gene is characterized by altered expression levels. This can result in a decrease or increase in the activity of vitamin D in cells. Many studies have described the opposing effects of polymorphic forms of the VDR gene on cancer risk, possibly due to the different numbers of patients examined and study sites of cancer, population origin, or applied statistical methods [12].Notably, the absence of a significant association does not dismiss the potential relevance of VDR gene polymorphisms altogether. Instead, it highlights the complexity of the genetic architecture underlying NPC and encourages the exploration of additional genetic markers and pathways. Furthermore, the multifactorial nature of NPC etiology implies the involvement of numerous genetic variations, each with modest effects that may require larger sample sizes to reach statistical significance.

Although our study did not establish a direct link between VDR bsmI and fokI gene polymorphisms and NPC risk, it contributes to the cumulative body of knowledge in this field. This negative result can guide future research directions, potentially steering investigations toward interactions between these polymorphisms and other genetic or environmental factors, epigenetic modifications, or viral infections that have been associated with NPC development.

4. Conclusions

In conclusion, our study reveals no association between VDR bsmI and fokI gene polymorphism and nasopharyngeal carcinoma in the Minangkabau ethnic group. While our study did not identify a significant association between VDR gene bsmI and fokI polymorphisms and the risk of nasopharyngeal carcinoma, this negative finding offers valuable insights into the intricate genetic determinants of NPC susceptibility. This study serves as a stepping stone for further research endeavors, encouraging collaborative efforts to unravel the multifaceted factors contributing to the development of nasopharyngeal carcinoma.

Conflict of Interest

The author reports no conflicts of interest in this work.

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Ethical statement

The study was approved by the Faculty of Medicine Andalas University ethics committee, Padang Indonesia (No.569/UN.16.2/KEP-FK/2022), and written informed consent was obtained from participants in both NPC and control groups.

Availability of data and material

We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Code availability

Not applicable

Consent to participate

All authors participated in this research study.

Consent for publication

All authors submitted consent to publish this article research in IJCBS.

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