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Evaluation of the Relationship Between Vitamin D Receptor Gene Polymorphism and Head and Neck Squamous Cell Carcinoma

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ABSTRACT

Objective: The paradoxical relationship between cancer and vitamin D has been known since 1990. Vitamin D receptor (VDR) is found in several tissues and, in contrast to popular belief, it is not only responsible for calcium and phosphorus metabolism but also associated with several other metabolic events. We conducted this study to investigate the effects of these factors on head and neck cancers by examining vitamin D, calcium, and phosphorus levels and polymorphisms of the VDR genes FokI and Bsm in patients with head and neck cancer.
Material and Methods: A total of 51 patients with head and neck squamous cell carcinoma aged 31-88 years and 51 healthy individuals aged 33-89 years who applied to the otorhinolaryngology outpatient clinic of Mersin University Faculty of Medicine were included in this study.
Results: No difference was observed in the distribution of the genotype ratios of the VDR genes FokI and Bsm between the two groups. However, a statistically significant difference was found in calcium levels between the two groups.
Conclusion: An individual with known VDR gene polymorphisms can provide possible risk information regarding which disease risk group he/she is in, and VDR gene polymorphism can be used as a biomarker.
Keywords: Vitamin D, genes, neoplasms, calcium, phosphorus, real-time polymerase chain reaction

INTRODUCTION

Head and neck cancers can be found in a wide range of anatomical areas, including the salivary glands, parts of the upper aerodigestive system, and parts of the ear (1).

Several studies conducted after 1990 found important evidence of an inverse relationship between cancer and vitamin D (2). After calcitriol enters the cell, it clenches the vitamin D receptor (VDR), creating an active complex that subsequently binds to nuclear chromatin, viz., the area on the DNA known as the vitamin D response element (VDRE) (3). VDR is one of the factors responsible for regulating vitamin D transcription. The human VDR gene, which is located on chromosome 12q12-q14, is a protein of 50 kD, consisting of 427 amino acids. Four different polymorphisms of VDR gene have been identified, viz., *FokI*, *Bsm*, *ApaI*, and *TaqI* (4).

The hematopoietic and immune system; heart, skeletal, and smooth muscle tissues; brain; liver, breasts, endothelium, skin (keratocytes, melanocytes, and fibroblasts), and endocrine glands (pituitary gland, parathyroid glands, and pancreatic islet beta cells); adrenal cortex and medulla thyroid; and ovaries and testicles are among the tissues with VDRs. In this manner, the antiproliferative effect of $1,25(OH)_2D$ responds to malignancies developed in these tissues (5).

Polymorphism is used to determine risk levels in people for diseases such as cancer, coronary heart disease, and diabetes and for the prenatal diagnosis of genetic diseases and detecting

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heterozygous carriers, tissue typing for organ transplantation, paternity testing, and forensic studies. In this regard, polymorphism is currently defined as a genetic marker (6).

Polymorphism of VDR genes is considered effective to determine cancer development or progression. VDR plays an important role in cell signaling pathways and cellular interactions that result in cancer progression (7).

A positive correlation has been found between *FokI* polymorphism and breast cancer. A comparative study in Europe involving 5,284 cases and 7,500 controls showed a significant increase in breast cancer risk in subjects with *FokI* polymorphism (8). It has been believed that inherited VDR variants exert an impact on cancer risk. Furthermore, a relationship may exist between the disease stage and survival rate and VDR polymorphism in patients with lung malignant neoplasm (small cell) (9). Another study reported an increase in the 245th codon mutation rate and VDR gene polymorphism in the Ff/ff genotype (10).

One study of patients with prostate cancer reported that calcium sensor receptor, estrogen receptor alpha, and VDR polymorphisms exert an effect on carcinogenesis and proliferation in prostate glands. Polymorphism of the VDR gene *Bsm* and especially the Bb/BB genotype were found to increase the risk of developing prostate cancer (11).

Regarding VDR, calcium sensor receptor polymorphism, and colorectal cancer risk, despite research on polymorphisms of the VDR genes *FokI* and *Bsm*, and although no relationship was observed between *FokI* and colorectal cancer, it was reported that a relationship might exist between *Bsm* polymorphism and colorectal cancer risk (12). A study conducted in Japan demonstrated that calcium and vitamin D levels were associated with breast malignancies in postmenopausal and premenopausal women (13). Another study reported that calcium, vitamin D, and retinol exert an effect on pancreatic cancer (14).

Consumption of 1,000–2,000 IU/day to reach at least 30 ng/ml of vitamin D, provided that daily calcium intake is not more than 1,000 mg/day, was found to be effective in preventing colorectal cancer (15).

We conducted this study to identify the possible relationships between VDR gene polymorphisms and head and neck squamous cell cancers.

MATERIAL and METHODS

The study sample consisted of 102 participants, with 51 individuals (patient group, Group 1, 8 women and 43 men, age 31–88 years) diagnosed with head and neck squamous cell carcinoma, who applied to the otorhinolaryngology outpatient clinic of Mersin University Medical Faculty Hospital, and 51 individuals (control group, Group 2, 9 women and 42 men, age 33–89 years) who have never been diagnosed with head and neck squamous cell carcinoma and do not have a history of any systemic disease.

When selecting the control group, key demographic factors such as age, gender, and geographical location were considered, providing a baseline for comparative analysis against the patient group.

Peripheral venous blood samples of the participants were collected into EDTA tubes, and their sera samples were collected into uncontained biochemistry tubes for separation.

Vitamin D3 levels in the blood samples collected in EDTA tubes were determined using the Cobas 800 autoanalyzer at the medical biochemistry laboratory of Mersin University. The

Table 1: Gender distribution of patient and control groups

Region	Number of people	%	Male/Female ratio
Larynx	31	60.78	14.5/1
Lip	4	7.84	4/0
Tongue	3	5.89	2/1
Retromolar Trigone	3	5.89	1/2
Parotid gland	2	3.92	1/1
External auditory canal	1	1.96	0/1
Paranasal Sinuses	1	1.96	1/0
Tongue Base + epiglottis	1	1.96	1/0
Squamous cell carcinoma of unknown primary in the head and neck (supraclavicular area)	1	1.96	1/0
Buccal	1	1.96	0/1
Floor of mouth	1	1.96	1/0
Nasal vestibule	1	1.96	1/0
Gingiva and alveolar arch	1	1.96	0/1

Table 2: Distribution of VDR *FokI* polymorphisms among genotypes in patient and control groups

			Group		Total
			Control	Patient	
FokI	Allele C	Number of people	24	24	48
		% *FokI*	50.0%	50.0%	100.0%
		% In groups	48.0%	47.1%	47.5%
		% Total	23.8%	23.8%	47.5%
	Allele T	Number of people	6	6	12
		% *FokI*	50.0%	50.0%	100.0%
		% In groups	12.0%	11.8%	11.9%
		% Total	5.9%	5.9%	11.9%
	Heterozygous	Number of people	20	21	41
		% *FokI*	48.8%	51.2%	100.0%
		% In groups	40.0%	41.2%	40.6%
		% Total	19.8%	20.8%	40.6%
Total		Number of people	50	51	101
		% *FokI*	49.5%	50.5%	100.0%
		% In groups	100.0%	100.0%	100.0%
		% Total	49.5%	50.5%	100.0%

Table 3: Distribution of VDR *Bsm* polymorphisms among genotypes in patient and control groups

			Group		Total
			Control	Patient	
Bsm	Allele G	Number of people	25	26	51
		% *Bsm*	49.0%	51.0%	100.0%
		% In groups	50.0%	51.0%	50.5%
		% Total	24.8%	25.7%	50.5%
	Allele A	Number of people	8	10	18
		% *Bsm*	44.4%	55.6%	100.0%
		% In groups	16.0%	19.6%	17.8%
		% Total	7.9%	9.9%	17.8%
	Heterozygous	Number of people	17	15	32
		% *Bsm*	53.1%	46.9%	100.0%
		% In groups	34.0%	29.4%	31.7%
		% Total	16.8%	14.9%	31.7%
Total		Number of people	50	51	101
		% *Bsm*	49.5%	50.5%	100.0%
		% In groups	100.0%	100.0%	100.0%
		% Total	49.5%	50.5%	100.0%

blood samples in the EDTA tubes were stored at +4°C until DNA isolation. Calcium and phosphorus levels of the serum, which was separated by centrifugation at 3,000 rpm for 600 s, and peripheral venous blood samples were kept in frameless biochemistry tubes for 600 s and then examined in the Cobas 400 autoanalyzer.

Statistical analysis

Statistical analyses were conducted using the STATISTICA 7.0 package program. Numerical data were subjected to the Kolmogorov–Smirnov normality test. Independent t-test and Mann–Whitney U test were used for comparing two groups.

Table 4: Distribution of VDR *FokI* polymorphisms between genotypes and biochemical parameters in patient and control groups

FokI	Group		Number	Mean	Standard deviation
Allele C	Control	Ca	24	12.872	8.487
		D3	24	14.589	10.156
		Valid N	24		
	Patient	Ca	24	9.013	0.752
		D3	24	11.718	5.064
		Valid N	24		
Allele T	Control	Ca	6	20.848	11.041
		D3	6	9.950	1.413
		Valid N	6		
	Patient	Ca	6	9.036	0.160
		D3	6	16.658	9.716
		Valid N	6		
Heterozygous	Control	Ca	20	10.354	4.564
		D3	20	14.994	9.917
		Valid N	20		
	Patient	Ca	21	9.417	0.540
		D3	21	15.316	9.529
		Valid N	21		

Table 5: Distribution of VDR *Bsm* polymorphisms between genotypes and biochemical parameters in patient and control groups

Bsm	Group		Number	Mean	Standard deviation
Allele G	Control	Ca	25	12.098	6.432
		D3	25	10.421	4.550
		Valid N	25		
	Patient	Ca	26	9.235	0.565
		D3	26	13.661	6.780
		Valid N	26		
Allele A	Control	Ca	8	15.233	12.480
		D3	8	18.011	11.815
		Valid N	8		
	Patient	Ca	10	9.345	0.834
		D3	10	9.982	4.783
		Valid N	10		
Heterozygous	Control	Ca	17	12.752	8.015
		D3	17	17.947	11.699
		Valid N	17		
	Patient	Ca	15	8.983	0.643
		D3	15	16.715	9.924
		Valid N	15		

Chi-square independence test statistics were used for nominal and categorical data. Descriptive statistics were also presented and considered statistically significant when p≤0.05.

This study was approved by the Clinical Research Ethics Committee of Mersin University (Date: 12.02.20215, No:38).

RESULTS

The gender distribution of the 51 patients with head and neck cancer according to the site of cancer is shown in Table 1.

There were 43 men and eight women in control patients, 42 men and nine women in the patient group. The mean age of subjects in the control group was 61.59±12.766 years, and that of subjects in the patient group was 64.49±11.231 years, with no significant difference in terms of age distribution (p=0.200).

Serum Ca levels did not show a normal distribution in the control group (p=0.001) but showed a normal distribution in the patient group (p=0.200), with differences being observed between the study groups (p=0.001). Serum phosphorus levels exhibited a normal distribution in the control group (p=0.200) but not in the patient group (p=0.040); however, there was no difference in phosphorus levels between the groups (p=0.864). Vitamin D3 levels showed a normal distribution in both groups (p=0.001 in the control group, p=0.019 in the patient group), with no difference being detected between the groups (patient vs control p=0.848).

Tables 2 and 3 show the percentage distribution of polymorphisms of the VDR genes *FokI* and *Bsm* among the genotypes in the study groups. There was no difference between the groups in the genotype ratio distribution of *FokI* (p=0.903) and *Bsm* (p=0.837). No difference was observed in the relationship between the groups in terms of *FokI* (p=0.993) and *Bsm* (p=0.754).

VDR *FokI*: The CC genotype rate was 47.1% in Group 1 and 48% in Group 2. The heterozygous (CT) genotype rate was 41.2% in Group 1 and 40% in Group 2. The TT genotype rate was 11.8% in Group 1 and 12% in Group 2.

VDR *Bsm*: The AA genotype rate was 19.6% in Group 1 and 16% in Group 2. The heterozygous (GA) genotype rate was 29.4% in Group 1 and 34% in Group 2. The GG genotype rate was 51% in Group 1 and 50% in Group 2.

No difference was detected between the groups in terms of the percentage distributions of allele C, allele T, and heterozygous genotype (p=0.903). Ca levels were different between the groups in individuals carrying the T allele (p=0.034). Vitamin D levels were different between groups in individuals carrying the A allele (p=0.028). Tables 4 and 5 show the distribution of polymorphisms of the VDR genes *FokI* and *Bsm* between genotypes and biochemical parameters.

DISCUSSION

VDR mediates the activity of vitamin D and participates in a variety of processes, including the regulation of cell proliferation and differentiation in normal tissues and cell death and cell adhesion in neoplastic cells. VDR gene and its polymorphism and vitamin D levels may be associated with malignancy. Vitamin D possesses extremely strong antineoplastic features. Calcitriol is an anti-reproductive and prodifferentiation factor for several cell types, including human squamous cells. *FokI* and *Bsm* are several polymorphisms of the VDR gene (16).

Gandini et al. investigated the relationship between vitamin D levels and gastrointestinal, endocrine, and genitourinary cancer (colorectal, breast, and prostate cancer) and observed an inverse relationship between vitamin D levels and colorectal cancer risk. An insignificant decrease in the risk of developing breast malignant tumors was found, but other studies have detected no relationship between vitamin D levels and breast cancer. Moreover, no relationship was detected between vitamin D levels and prostate cancer (17, 18).

Polymorphisms in the gene encoding VDR may exert an effect on cancer risk (19). Although numerous studies have analyzed VDR polymorphisms, their general relationship with carcinogenesis remains controversial. Significant relationships have been reported between VDR gene polymorphisms and breast (*FokI*, *Bsm*, and *ApaI*), prostate (*FokI*, *Bsm*, and *TaqI*), colorectal (*FokI*, *Bsm*, and *TaqI*), and skin (*FokI*, *Bsm*, *TaqI*) (20) cancers. There is only one study in the English literature on the relationship between head and neck cancers and polymorphism of the VDR gene *FokI*, whereas there is no study on *Bsm*. Liua et al. examined 719 patients with otorhinolaryngological squamous cell carcinoma for VDR polymorphism (FokI ff homozygous) in Texas and found a difference in polymorphism between patient and control groups. However, in our study, there was no relationship between VDR gene polymorphisms (*FokI* and *Bsm*) and head and neck squamous cell carcinoma (21). This difference between our study and that by Liua et al. may be due to difference in the number of study subjects.

A study by the European Prospective Investigation into Cancer and Nutrition (EPIC) demonstrated that patients who have an adequate level of vitamin D concentration in their blood circulation have a reduced risk of developing head and neck cancer and exhibit a higher rate of survival after diagnosis (22). A high phosphorus content is responsible for tumor growth and progression (23, 24).

In our study, no difference was detected between Group 1 and Group 2 in the levels of vitamin D (control: 14.110±9.374, patients: 13.90±7.656) and phosphorus (control: 3.405±0.628, patients: 3.441±0.785). These differences may be related to environmental factors (exposure to sunlight, nutrition). Hypocalcemia occurs in approximately 30% of all patients diagnosed with prostate cancer (25).

CONCLUSION

Hypocalcemia was observed in 15.68% of the patients. When calcium levels and genotype distributions of *FokI* polymorphisms were examined, individuals with *FokI* T allele and patients with HNSCC have lower blood calcium levels

than subjects in a randomly selected group. A comparison of calcium levels and *FokI* polymorphism alleles in our total sample of 102 participants revealed that individuals with the T allele have lower levels of calcium. When the *Bsm* VDR gene polymorphism was examined, the vitamin D level showed a statistically significant difference in individuals with the A allele. The different results obtained in studies on the relationship between *FokI* and *Bsm* polymorphisms and patients with HNSCC in different ethnic populations are not yet sufficient to explain the pathophysiological mechanism of the event. Our findings indicate that the investigated biochemical parameters (vitamin D, phosphorus, and calcium) are associated with the activity of VDR gene polymorphisms (*FokI* and *Bsm*). Confirmation of these findings through larger studies by including more participants and all exons, introns, and promoter regions Finding the VDR gene would make this study highly valuable.

Although the sample size for this study was determined based on available resources and time constraints, we acknowledge that future studies using larger sample groups may further confirm our findings. The results of our study differ from some findings in the literature. We believe that these discrepancies arise from variations in the genetic diversity of study populations, methodological approaches, or the specific polymorphisms analyzed. Our study did not extensively investigate environmental and lifestyle factors that could affect vitamin D levels and VDR gene polymorphisms. Future research should consider factors such as sun exposure, dietary habits, and smoking status. The specific biological mechanisms by which VDR gene polymorphisms might affect the risk of developing HNSCC remain unclear; however, considering the role of VDR in regulating cell growth and differentiation, these gene variations could be hypothesized to play a significant role in cancer development.

According to our results, an individual with known VDR gene polymorphisms can be informed about which disease risk group he/she is in, and VDR polymorphisms can be used as a bioindicator. Therefore, by being aware of their risk group in advance, individuals can have a better quality of life by making changes in their lifestyle and diet.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Mersin University (Date: 12.02.20215, No:38).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Data Acquisition- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Data Analysis/Interpretation- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Drafting Manuscript- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Critical Revision of Manuscript- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Final Approval and Accountability- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Material or Technical Support- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.; Supervision- S.C., Z.N.Ü., K.K.B., H.G., O.İ., M.Ü.

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