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Genetic Association Of Vitamin D Receptor (Rs731236) Gene Polymorphism With Susceptibility To Oral Cancer In The South Indian Population – A Case-Control Study

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ABSTRACT

Background: *Oral cancer poses a significant public health challenge, particularly in regions with high tobacco and betel quid consumption. Despite advances in treatment, prognosis remains poor due to late-stage diagnosis and multifactorial etiology. Recent research suggests a potential role for vitamin D and its receptor (VDR) in cancer prevention and treatment.*

Aim: *To determine the genetic association of the VDR (rs731236) polymorphism with susceptibility to oral cancer in the South Indian population.*

Materials and Methods: *A case-control study was conducted with 50 participants (25 cases with oral squamous cell carcinoma [OSCC] and 25 controls). Genomic DNA was isolated from peripheral blood leukocytes, and genotyping of the VDR (rs731236) polymorphism was performed using PCR-ARMS method. Statistical analysis was conducted to assess genotype frequencies, Hardy-Weinberg equilibrium, and association with oral cancer susceptibility.*

Results: *Demographic characteristics of participants were similar between cases and controls. Genotype frequencies of the VDR (rs731236) polymorphism were in Hardy-Weinberg equilibrium and did not differ significantly between cases and controls. Allele frequencies were comparable to*

those reported in other populations. There was no significant association between the VDR (rs731236) polymorphism and oral cancer susceptibility in the South Indian population.

Conclusion: *This study found no significant genetic association between the VDR (rs731236) polymorphism and susceptibility to oral cancer in the South Indian population. Further research with larger sample sizes and diverse populations is warranted to clarify the relationship between VDR polymorphisms and oral cancer risk, which is essential for the development of effective prevention and treatment strategies.*

Keywords: Vitamin D, VDR polymorphism, OSCC

1.1 INTRODUCTION

Oral cancer is a major public health issue worldwide, particularly in regions with high consumption of tobacco and betel quid, such as South Asia. Oral Squamous Cell Carcinoma (OSCC) is the most common type of oral cancer, accounting for more than 90% of all cases [1]. The prognosis of OSCC remains poor despite advances in treatment modalities, largely due to late-stage diagnosis and the complex etiology involving genetic, environmental, and lifestyle factors [2].

Recent studies have highlighted the potential role of vitamin D and its receptor in cancer prevention and treatment. Vitamin D is a fat-soluble vitamin that is synthesized in the skin upon exposure to ultraviolet B (UVB) radiation from sunlight. It is then metabolized in the liver and kidneys to its active form, calcitriol, which exerts its effects by binding to the Vitamin D Receptor (VDR). The VDR is a member of the nuclear receptor superfamily, encoded by a gene located on chromosome 12q12-14 [3]. This receptor is present in various tissues, including the skin, bone, and numerous tumor tissues, indicating its potential role in cellular differentiation, proliferation, and apoptosis [4].

The potential role of vitamin D in cancer prevention is supported by several epidemiological studies that have observed an inverse relationship between sunlight exposure, serum vitamin D levels, and the risk of various cancers, including colorectal, breast, and prostate cancers [5-7]. Mechanistically, vitamin D is known to induce cell cycle arrest, promote differentiation, and trigger apoptosis in cancer cells [8-10]. In the context of oral cancer, the induction of apoptosis in tumors and Oral Potentially Malignant Disorders (OPMDs) that express VDR, such as oral lichen planus and leukoplakia, through vitamin D could be beneficial for chemoprevention and as an adjunctive treatment strategy [11-13].

The VDR gene harbors numerous Single Nucleotide Polymorphisms (SNPs), which have been investigated for their association with cancer susceptibility. Notably, polymorphisms such as FokI, BsmI, TaqI, and ApaI have been studied in various cancers [14-16]. The TaqI polymorphism, an intronic variant, is of particular interest due to its role in transcription factor binding and its linkage disequilibrium with other functional genes, which may indirectly influence disease susceptibility [17,18]. However, no studies to date have examined the association of the VDR (rs731236) polymorphism with oral cancer susceptibility in the South Indian population, prompting the need for this investigation. The aim of this study is to determine the genetic association of the Vitamin D Receptor (rs731236) gene polymorphism with susceptibility to oral cancer in the South Indian population.

1.2 MATERIALS AND METHODS

1.2.1 Study Design and Participants

A case-control study was conducted with a total of 50 participants, including 25 OSCC cases and 25 controls. The sample size calculation was done using G-power analysis by keeping the alpha error probability as 0.05, allocation ratio as 1 and the power of the study (1-beta error probability) as 0.99, the sample size was calculated as n =50. The protocol of the study was approved by the Scientific Review Board **(IHEC/SDC/FACULTY/23/OPATH/108)** and it conforms to the provisions of the declaration of Helsinki. The cases were patients diagnosed with OSCC, confirmed by histopathological examination [19]. Controls were age and sex-matched individuals without any history of oral cancer or other malignancies. Informed consent was obtained from all participants, and the study was approved by the institutional human ethical committee.

1.2.2 Sample Collection and DNA Extraction

Blood samples (5 ml) were collected from each participant using EDTA as an anticoagulant. Genomic DNA was isolated from peripheral blood leukocytes using a standard phenol-chloroform extraction method [20]. The quality and quantity of the extracted DNA were assessed by spectrophotometry and agarose gel electrophoresis [21].

1.2.3 Genotyping

Genotyping of the VDR (rs731236) polymorphism was performed using the PCR-ARMS (Polymerase Chain Reaction - Amplification Refractory Mutation System) method [22]. The primers used for amplification were:

- Forward: 5'-ATGGAAGGACCTAGGTCTGGAT-3'
- Reverse: 5'-TTCTGGATCATCTTGGCATA-3' The expected amplicon size was 344 bp.

1.2.4 PCR Conditions and Gel Electrophoresis

The PCR reactions were carried out in a total volume of 25 µl containing 50 ng of genomic DNA, 10 pmol of each primer, 1X PCR buffer, 1.5 mM MgCl2, 200 µM dNTPs, and 1 U of Taq DNA polymerase. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes [23].

The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. The presence of specific bands corresponding to the T and C alleles was used to determine the genotypes [24].(Fig.1, Fig.2)

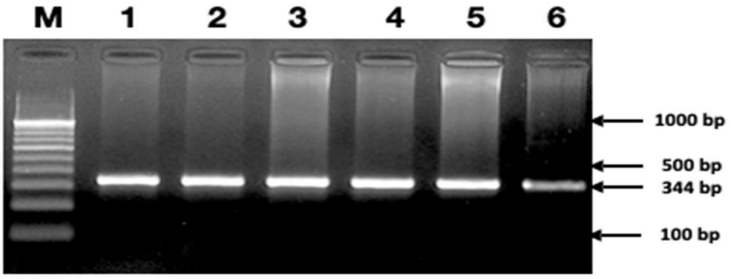

Figure 1: Agarose gel electrophoretogram showing partial amplification of Vitamin D receptor (*rs731236)* spanning site run along with standard DNA ladder [Lane M = 100 bp DNA marker].

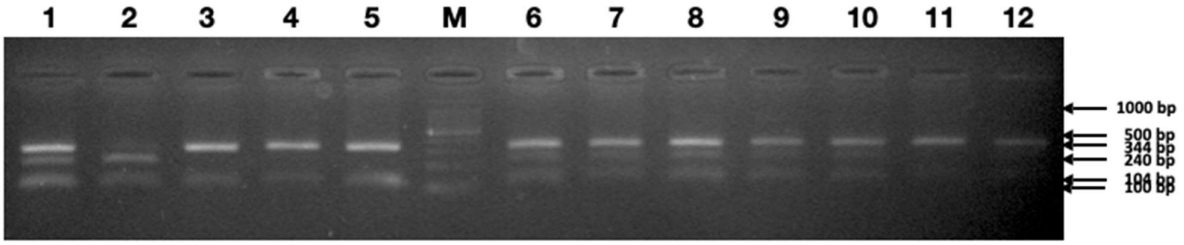

Figure 2: Agarose gel electrophoretogram showing TaqI digested amplicon of VDR (rs731236) with the following genotypes: Homozygous TT (344 bp), Heterozygous TC (344+242+102 bp), Homozygous CC (242+102 bp).

1.2.5 Statistical Analysis

Genotype frequencies were calculated for cases and controls, and the Chi-square test was used to assess Hardy-Weinberg equilibrium. The association between the VDR (rs731236) polymorphism and oral cancer susceptibility was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs). Statistical significance was set at $P < 0.05$. All analyses were performed using SPSS software (version 22.0).

1.3 RESULTS

1.3.1 Demographic Characteristics

The demographic characteristics of the study participants are summarized in Table 1. The mean age of the cases was 55.4 years (range: 40-70 years), and the mean age of the controls was 54.2 years (range: 42-68 years). There was no significant difference in the distribution of age, sex, or other demographic variables between cases and controls. (Table.1)

Table.1: Table showing the demographic details of all the participants involved in the study.

	Case	**Control**
Male	21	16
Female	4	9
Habits (Smoking/ Pan Chewing)	25	3
Alcohol	7	0
MDSCC	10	0
WDSCC	8	0

Other types	7	0

1.3.2 Genotype Frequencies and Hardy-Weinberg Equilibrium

The genotype frequencies of the VDR (rs731236) polymorphism among cases and controls are presented in Table 2. The distribution of genotypes in both groups was in Hardy-Weinberg equilibrium (P = 0.8344), indicating no deviation from expected frequencies in the population. (Table.2)

Table 2: Genotype frequencies of Vitamin D receptor (*rs731236)* among the cases and controls

Groups	TT	TC	CC	T	C	HWE (p-value)*
Case (N=25)	17	6	2	0.80	0.20	0.2113
Control (N=25)	18	6	1	0.84	0.16	0.5921

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom.
The genotype frequency do not differ between the cases and controls at χ2df (P =0.8344).

1.3.3 Association Analysis

The allele and genotype frequencies of the VDR (rs731236) polymorphism are shown in Figure 3 and Figure 4. The frequency of the T allele was 80% in cases and 84% in controls, while the C allele was present at a frequency of 20% in cases and 16% in controls. The genotype frequencies did not differ significantly between cases and controls (P = 0.8344). (Fig.3, Fig.4)

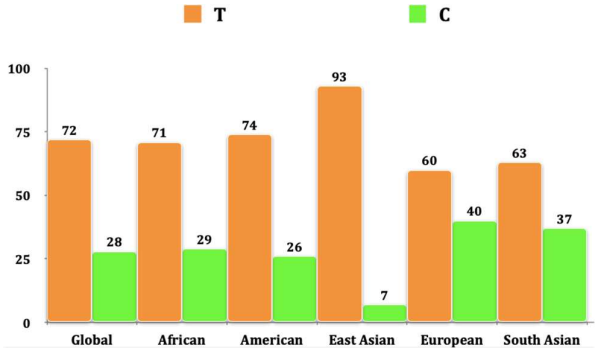

Figure. 3: The graph depicts the allele frequency of polymorphism in various study population (Ensembl database)

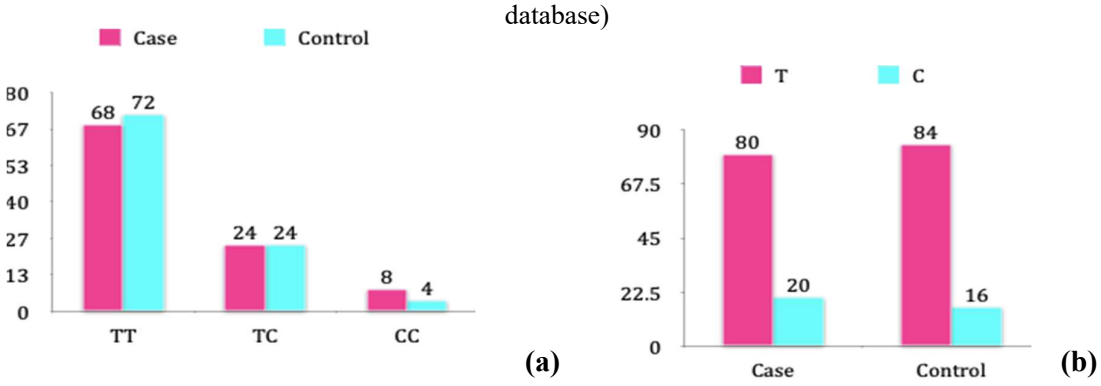

Figure. 4: The graph depicts the (a) genotype and (b) allele frequency of polymorphism in the present study group expressed as percentage.

1.4 DISCUSSION

The Vitamin D receptor (VDR), which plays a crucial role in cellular processes such as proliferation, differentiation, and apoptosis, has been extensively studied for its involvement in cancer. The VDR gene spans approximately 75 kb on chromosome 12q12-14 and contains several polymorphisms, including the TaqI polymorphism. This polymorphism has garnered attention for its potential role in cancer susceptibility, including oral cancer.

Vitamin D, in its active form calcitriol, binds to VDR and regulates the expression of numerous genes involved in cell cycle control, differentiation, and apoptosis. Calcitriol inhibits cancer cell proliferation by inducing cell cycle arrest at the G1 phase, promoting differentiation, and enhancing apoptosis by modulating various signaling pathways, including the p21, p27, and Bcl-2 pathways [25, 26]. Additionally, calcitriol's anti-inflammatory properties may contribute to its anticancer effects by reducing chronic inflammation that often accompanies tumor development [27].

The TaqI polymorphism (rs731236) is located in the intronic region of the VDR gene and is thought to influence the expression and function of the receptor by affecting transcription factor binding and mRNA stability [28]. Several studies have reported associations between the TaqI polymorphism and various cancers, including breast, prostate, and colorectal cancer [29-31]. However, findings have been inconsistent, likely due to differences in study design, sample size, and population characteristics.

Previous studies reported in the literature on the association between the TaqI polymorphism and oral cancer have produced mixed results. Some studies have reported a significant association, suggesting that the TaqI polymorphism may increase the risk of developing oral cancer [32, 33]. These studies hypothesize that the polymorphism affects VDR function, leading to altered cell cycle regulation and increased susceptibility to malignant transformation in oral tissues.

Our study aimed to investigate the genetic association of the VDR (rs731236) polymorphism with susceptibility to oral cancer in the South Indian population. Contrary to some previous study results, we did not observe a significant association between the VDR (rs731236) polymorphism and susceptibility to oral cancer in this population. The genotype frequencies of the polymorphism were similar between cases and controls, and the allele frequencies were comparable to those reported in

other populations, such as the American population [34, 35]. The lack of association in our study may be attributed to the small sample size, which limits the statistical power to detect a significant effect. Additionally, oral cancer is a multifactorial disease influenced by a combination of genetic, environmental, and lifestyle factors, and the contribution of a single polymorphism may be modest [36,37]. The main limitations of the present study are the small sample size, variations in the genetic background and environmental exposure such as UV radiation, diet, and tobacco use,which can modulate the impact of genetic polymorphisms on cancer risk.

Identifying genetic risk factors for oral cancer has significant clinical and public health implications. Understanding genetic predispositions can aid in developing personalized prevention and treatment strategies. For instance, individuals with high-risk genotypes may benefit from more frequent screenings and early detection efforts [37, 38]. Additionally, the modulation of vitamin D levels through supplementation or dietary interventions could be explored as a potential chemopreventive strategy for individuals at high risk of oral cancer [38]. However, further research is needed to establish the efficacy and safety of such interventions [39].

Future studies can be done by increasing the sample size, involving diverse population, to account for genetic diversity and environmental factors [39-40], large scale functional studies to understand the biological mechanisms by which VDR polymorphisms influence cancer risk, including their impact on gene expression, receptor function, and cellular processes. Also studies can be done to explore the interactions between genetic polymorphisms and environmental factors, such as UV exposure, diet, and tobacco use, in the context of oral cancer risk and to assess the potential of vitamin D supplementation and other preventive strategies in individuals with high-risk genotypes and evaluate the role of VDR-targeted therapies in the treatment of oral cancer.

1.5 CONCLUSION

The TaqI polymorphism in the VDR gene has been studied for its potential role in cancer susceptibility, including oral cancer. While some studies have reported associations between the TaqI polymorphism and increased cancer risk, our study did not find a significant association in a South Indian population. These discrepancies highlight the need for further research with larger sample sizes and diverse populations to better understand the role of VDR polymorphisms in oral cancer. Identifying genetic risk factors and understanding their mechanisms can inform personalized prevention and treatment strategies, ultimately improving clinical and public health outcomes.

1.6 STATEMENTS AND DECLARATIONS

1.6.1 Funding: No sources of funding declared.

1.6.2 Competing Interest: No competing interest or conflict of interest between the authors declared.

1.6.3 Author contribution: *All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Sandra Sagar and Dr. Vijayashree Priyadharshini. The first draft of the manuscript was written by Dr. Sandra Sagar, Dr. Pratibha Ramani and Dr. Genickson Jeyaraj and all authors commented on previous versions of the manuscript. Dr. Shamina Ross helped with the statistical analysis and final proofing of the manuscript. All authors read and approved the final manuscript.*

1.6.4 Ethics approval and consent to participate

Ethical Committee Clearance Number: (**IHEC/SDC/FACULTY/23/OPATH/108**)."The study was approved by the institutional human ethical committee board of Saveetha Dental College and Hospitals". The protocol of the study was approved by the Scientific Review Board and it conforms to the provisions of the declaration of Helsinki. An informed consent was obtained from all the patients by informing and clearly explaining the details of the study. All the methods in the study was performed in accordance to the relevant regulations and guidelines.

1.6.5 Consent to participate

An informed consent was obtained from all the patients by informing and clearly explaining the details of the study.

1.6.6 Consent for publication

An informed consent was obtained from all the patients and their legal guardians by informing and clearly explaining the details of the study.

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LEGENDS

Table.1: Table showing the demographic details of all the participants involved in the study.

Table 2: Genotype frequencies of Vitamin D receptor (*rs731236)* among the cases and controls

Figure 1: Agarose gel electrophoretogram showing partial amplification of the VDR (rs731236) spanning site along with a standard DNA ladder.

Figure 2: Agarose gel electrophoretogram showing TaqI digested amplicon of VDR (rs731236) with the following genotypes: Homozygous TT (344 bp), Heterozygous TC (344+242+102 bp), Homozygous CC (242+102 bp).

Figure.3: Allele frequency of polymorphism in various study populations.

Figure.4: Genotype and allele frequency of polymorphism in the present study group expressed as percentages.