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ORIGINAL ARTICLE

VDR-Fok I polymorphism and chronic periodontitis

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Association of vitamin D receptor gene polymorphism (*rs10735810*) and chronic periodontitis

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Abstract

Aim: The aim of the present study was to analyze the association between vitamin D receptor (*VDR*) (*rs10735810*) gene polymorphism and chronic periodontitis (CP).

Methods: A total of 100 subjects were recruited for this study, which included 50 CP and 50 healthy controls. Genomic DNA was extracted from the whole blood collected from the subjects. DNA was amplified using specific primers flanking the *FokI* region of the *VDR* gene (*rs10735810*). The amplicon was further subjected to genotyping using restriction fragment length polymorphism (RFLP) using the *FokI* enzyme. The genotype obtained based on RFLP pattern was recorded and used for statistical analysis. The distribution of genotypes and allele frequencies in the chronic periodontitis and control groups were compared using the χ^2-test.

Results: The CP group displayed the highest frequency of CT (20%) and TT (6%) genotypes when compared with the control subjects. Allele frequency was found to be similar in both groups. The C allele was found to be predominant in the study population compared with the T allele.

Conclusion: The present study denotes that the *VDR* polymorphism (*rs10735810*) is not associated with CP in the study group analyzed.

KEYWORDS

alleles, chronic periodontitis, FokI, polymorphism, vitamin D receptor

1 | INTRODUCTION

Periodontitis is a chronic inflammatory disease which has a multifactorial etiology. Although the presence of Gram-negative anaerobic bacteria is essential for the initiation of periodontal destruction, many environmental and genetic factors could result in the progression of the disease. The genetic influence plays a key role in determining the host susceptibility to periodontal destruction.[1,2] Many studies have attempted to identify the genetic factors that may be related to enhanced susceptibility to periodontal disease.[3-8]

Vitamin D (Vit D; 1.25-dihydroxyvitamin D_3) is a fat-soluble steroid hormone that interacts with its nuclear receptor, vitamin D receptor (*VDR*), to regulate different biological processes, such as bone metabolism and immunomodulatory response. On activation by parathormone (PTH), Vit D helps the intestines absorb calcium. It acts to increase the amount of calcium that the intestines can absorb by 2-4 times. For patients with Vit D deficiency, it is difficult for the body to obtain calcium from the diet. This often leads to a rise in the PTH level, because the parathyroid glands must increase the PTH production in order to increase the calcium levels by utilizing it from the bone.

The active form of Vit D (1,25 D) exerts its effect on the tissue by binding to the *VDR*. This complex dimerizes with the retinoid X receptor (RXR) and the 1,25 D-*VDR*-RXR heterodimer translocates to the nucleus where it binds Vit D-responsive elements (*VDR*E) in the promoter region of Vit D-responsive genes and induces the

expression of these Vit D-responsive genes.[9] Several studies have reported a positive association between osteoporosis and alveolar bone and tooth loss, which suggests that poor bone quality is a risk factor for developing periodontal disease.[10,11] *VDR* is a family of transcriptional regulatory factors and has a sequence similar to the steroid and thyroid hormone receptors.[12] *VDR* is encoded by the *VDR* gene, which contains 9 exons, spans approximately 75 kb, localized to chromosome 12q13.11 and expressed in the intestines, thyroid gland and kidneys.[13] Thus, when there is any gene alteration in the receptor responsible for Vit D it results in decreased production of calcium thereby causing increased osteoclast activity, bone loss and periodontal disease.

Vitamin D also plays an important part in innate antimicrobial response. Toll-like receptor binding leads to increased expression of both the 1-α-hydroxylase and the *VDR*.[14] This results in binding of the 1,25 D-*VDR*-RXR heterodimer to the *VDR*E of the gene for cathelicidin and β-defensin 4 and subsequent transcription of these proteins. This requires binding of nuclear factor-κB to appropriate response elements on the β-defensin 4 RNA. This affects the initial innate response by the host towards the bacteria and increases the amount of bacterial colonization in periodontitis.

Vitamin D inhibits B-cell proliferation, differentiation and immunoglobin secretion.[15] It also suppress T-cell proliferation and results in a shift from T-helper (Th)1 to Th2 phenotype. Vit D also affects T-cell maturation with a skewing away from the inflammatory Th17 phenotype. These effects result in decreased production of inflammatory cytokines (interleukin [IL]-17, IL-21) with increased production of anti-inflammatory cytokines such as IL-10. Vit D also has effects on monocytes and dendritic cells. It inhibits monocyte production of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and tumor necrosis factor-α.[16] In Vit D deficiency, genes responsible for the *VDR* are affected, altering the host response in cases of periodontal diseases and resulting in increased production of inflammatory cytokines leading to increased tissue destruction and altered bone metabolism, thereby resulting in periodontal disease.

Vitamin D receptor gene polymorphism and its susceptibility to chronic and aggressive periodontal diseases are studied using *TaqI*, *ApaI*, *BsmI* and *FokI* restriction enzymes.[17,18] *FokI* single nucleotide polymorphism (SNP) represents a missense mutation in the translation initiation site which modifies the length and functional activity of *VDR* protein. Tachi et al[19] showed an association between *VDR* gene polymorphism and periodontal disease. Many association studies have shown conflicting results of the relationship between *VDR* gene polymorphisms and susceptibility to chronic periodontitis.[20-22] Thus, the aim of the study was to compare the association of FokI *VDR* gene polymorphism with chronic periodontitis.

2 | MATERIALS AND METHODS

This study employed a cross-sectional design involving individuals from Chennai, Tamil Nadu, India. A total of 100 individuals who reported to the Department of Periodontics, Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N = 50) and CP group B (N = 50) based on the clinical examination of probing pocket depth, clinical attachment loss and bleeding on probing. The CP group contained 50 patients (26 male, 24 female) with a mean age of 39.02 ± 8.22 years. The CP patients were recruited based on the 1999 criteria of the American Academy of Periodontology.[23] The control group contained 50 periodontally healthy subjects (26 male, 24 female) with mean age of 41.34 ± 7.49 years.

A detailed history of dental treatment, family history of periodontal diseases, smoking habits as well as general health concerns were obtained from the subjects. Except for the presence of periodontitis, the patients included in this study were systemically healthy. Smokers, pregnant or lactating mothers, immunocompromised individuals and subjects who had undergone periodontal therapy within the past 6 months were excluded from this study. The study was approved by the institutional ethics committee (SRB/MDS/PERIO/18-19/0046).

2.1 | Sample collection and DNA extraction

A volume of 5 mL of venous blood was collected from the antecubital fossa and dispersed in a sterile tube containing a pinch of ethylenediaminetetraacetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller et al 1998 protocol.[24]

2.2 | Polymerase chain reaction and restriction endonuclease digestion

Vitamin D receptor gene (FokI) polymorphisms were assessed by polymerase chain reaction (PCR) amplification and restriction digestion. The primers forward 5′-AGCTGGCCCTGGCACTGACTCTGGCT-3′ and reverse 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′ were used for amplification of DNA spanning the *FokI* polymorphic site of the *VDR* gene. The amplification of DNA was performed in 20-μL volumes using 10 ng of genomic DNA, 5 pmol/μL each of the forward and reverse primers along with PCR Master Mix (Takara, Shiga, Japan). The cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 35 seconds, annealing at 60°C for 35 seconds, extension at 72°C for 35 seconds and a final extension at 72°C for 5 minutes. A 5-μL volume of PCR product was checked on a 1% agarose gel, and 15 μL of PCR product was digested using a *FokI* restriction enzyme (New England Biolabs, Hitchin, UK). Digestion was carried out at 37°C for 2 hours. The digested product was visualized on 2% agarose gel and the results were documented.

2.3 | Statistical analysis

All statistical analyses were performed using SPSS version 23.0 for Windows (SPSS, Chicago, IL, USA). The distribution of genotypes and allele frequencies in the chronic periodontitis and control groups

were compared using the χ^2-test. The risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence intervals. Statistical significance in all tests was set at $P < .05$.

3 | RESULTS

The clinical characteristics of the subjects in the CP and control groups are shown in Table 1. The genotype frequency and distributions of the groups are shown in Tables 2 and 3. Allele frequency of *VDR* polymorphism (*rs10735810*) in various geographic regions as acquired from the Ensembl genome browser (https://asia.ensembl.org) is represented in Table 4. The genotype frequency of *VDR* FokI polymorphism did not differ significantly at χ^{2df} ($P = .750$). Our study results showed that the prevalence of homozygous and heterozygous mutant genotypes had no significant difference (CC vs CT + TT) between the CP and healthy control group with a *P*-value of .4769. The detected frequency of CT (20% vs 16%) and TT (6% vs 4%) genotypes showed no significant difference between the CP group and healthy control subjects. There was no significant difference in C allele (84% vs 88%) and T allele (16% vs 12%) between the CP and healthy control group.

TABLE 4 Frequency of vitamin D receptor polymorphism (*rs10735810*) in various geographic regions

Population	Allele T	Allele C
African	19	81
American	48	52
East Asian	42	58
European	42	58
South Asian	26	74
Overall	33	67

TABLE 1 Data of chronic periodontitis and control groups

Clinical characteristics	Chronic periodontitis group			Control group		
	Male	Female	Total	Male	Female	Total
No. of subjects	26	24	50	26	24	50
Mean age, years	39.02 ± 8.22			41.34 ± 7.488		
Clinical attachment loss	6.13 ± 1.29			—		
Probing pocket depth	5.48 ± 1.15			1.60 ± 0.57		
Gingival index	1.74 ± 0.22			0.76 ± 0.16		

TABLE 2 Genotype frequencies of *VDR* (*rs10735810*) polymorphism among cases and controls

Genotypes	CC	CT	TT	Allele frequency	HWE, P^*
Cases (N = 50)	37 (74%)	10 (20%)	3 (6%)	C = 0.84 T = 0.16	.07
Controls (N = 50)	40 (80%)	8 (16%)	2 (4%)	C = 0.88 T = 0.12	.08

The genotype frequency of cases and controls do not differ significantly χ^{2df} ($P = .750$).
*For departure from Hardy-Weinberg equilibrium (HWE), chi square with 1 *df*.

TABLE 3 Distribution of the *VDR* (*rs10735810*) polymorphism among cases and controls

Models	Cases N = 50 (%)	Controls N = 50 (%)	Unadjusted OR (95% confidence interval)	*P*
Dominant				
CC	37	40	0.7115 (0.2785-1.8176)	.4769
CT + TT	13	10		
Recessive				
TT	3	2	0.6528 (0.1043-4.0851)	.6485
CT + CC	47	48		
Additive				
C	84	88	0.7159 (0.3198-1.6029)	.4164
T	16	12		

4 | DISCUSSION

Genetic polymorphisms, like SNP, may influence disease in multiple complex ways, acting with other genetic variants and environmental factors to influence disease susceptibility and progression. Many studies have revealed that SNP may be associated with susceptibility to periodontitis.[1] Human *VDR* is a ligand-regulated transcription factor that mediates the actions of the 1,25-dihydroxy vitamin D_3 hormone to effect bone mineral homeostasis. The *VDR* mediates the hormonal function of Vit D and regulates a variety of downstream functions.

Our study results showed that the genotype frequency of *VDR FokI* polymorphism did not differ significantly at χ^{2df} (P = .750). The prevalence of homozygous and heterozygous mutant genotypes showed no significant difference (CC vs CT + TT) between the CP and healthy control group with a *P*-value of .4769. The detected frequency of CT (20% vs 16%) and TT (6% vs 4%) genotypes had no significant difference between the CP group and healthy control subjects. There was no significant difference in C allele (84% vs 88%) and T allele (16% vs 12%) between the CP and healthy control group.

El Jilani et al[25] reported that there was no significant association between CP and *FokI* A/G SNP *rs2228570* in a Libyan population which was in accordance with our present study. A study performed by Arroyave et al[26] in a Colombian population showed that although the *rs2228570* (*FokI*) polymorphism remains a potentially functional variant, there was no association either between the different genotype/allele frequencies or haplotype and CP; it does not appear to be in linkage disequilibrium with the *BsmI*, *ApaI* or *TaqI* polymorphic sites, which was in accordance with our study.

However, a study performed by Chantarangsu et al[27] in a Thai population showed that CC + CT genotypes of *FokI* polymorphism were associated with severe CP with an OR of 1.9. Although association studies relating polymorphisms in this gene to periodontitis have been published, results are conflicting, possibly because of variations in study design, sample sizes and heterogenous populations, among other issues. Accumulating evidence from basic and clinical research makes the association between *VDR* gene polymorphism and periodontal disease. *VDR* gene polymorphism has been strongly associated with bone mineral density in many studies. Some investigators presumed that the *VDR* polymorphisms influence bone resorption and immune function.[28,29]

4.1 | Conclusion

Thus, our study concludes that the *VDR* gene *FokI* (*rs10735810*) polymorphism had no significant association in chronic periodontitis. Further multicentered studies are needed to achieve a better understanding of *VDR* gene polymorphism among various populations.

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