Analysis of Vitamin D Receptor Gene Polymorphism in Chronic Periodontitis among Saudi Population

Fathy M. Elfasakhany[1,2] Omaima N. Al-Qahtani[3] Asmaa M. Badri[3] Hala A. Abuelela[4]

[1]Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia
[2]Department of Medical Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt
[3]Dental Intern Program, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia
[4]Department of Periodontology and implant Dentistry, Faculty of Dentistry, Ain Shams and Misr International Universities, Egypt

Address for correspondence F.M. Elfasakhany, Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Abdia 715, Saudi Arabia (e-mail: fmfasakhany@uqu.edu.sa).

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Abstract

Objective Genetic and environmental factors have important roles in the development of periodontitis. We aimed to assess the relation of vitamin D receptor (VDR) *Apa*I and *Taq*I polymorphisms and the susceptibility of periodontitis in Saudi population in Makkah region.

Materials and Methods In total, 86 unrelated patients with moderate-to-severe periodontitis and 86 controls were enrolled in this study. Evaluation of the periodontal state was performed by using plaque index, bleeding on probing, probing depth, and attachment loss. Extraction of genomic DNA from peripheral blood and genotyping of VDR gene ApaI G/T (rs7975232) and *Taq*I T/C (rs731236) polymorphisms were performed by utilizing polymerase chain reaction and restriction digestion.

Results There were statistically significant differences between both groups regarding the mean bleeding on probing, mean probing depth, mean plaque index, and the mean attachment level ($p < 0.001$) indicating that the matching based on the investigated groups was adequate. The examined populations were in Hardy–Weinberg equilibrium. Analysis of the genotype and allele frequencies of both VDR *Apa*I and *Taq*I single nucleotide polymorphisms revealed that they were statistically indifferent between the control group and the periodontitis subjects ($p > 0.05$).

Conclusion These results suggested that VDR *Apa*I and *Taq*I polymorphisms might not be related to the susceptibility of periodontal disease in the Saudi subjects in Makkah region.

Keywords
- *Apa*I
- *Taq*I
- gene polymorphism
- periodontitis
- vitamin D receptor
- polymerase chain reaction

Introduction

Periodontal disease is a chronic infectious condition of the teeth surrounding tissues and is often reported as a multifactorial ailment with gradual progression. The phenotype of this condition is determined by both hereditary and environmental elements.[1-4] The most remarkable etiologic and scientific criteria of the condition are the formation of microbial plaque, inflammation of the periodontium, and damage of the supporting ligament and alveolar bone.[5] The rate of occurrence and progression of the condition increase with the age and generally affects each gender

equally. Additionally, periodontal disease is frequently seen among families, indicating a genetic predisposition to the condition.[6,7] Moreover, numerous studies reported the role of several gene polymorphisms in periodontitis as the gene polymorphism may lead to change in the structure or expression of the gene product, and this may alter the innate and adaptive immunity which will determine the disease outcome.[8] As long as the resorption of the alveolar bone is a prominent feature of periodontitis, it is reasonable that regulators of bone metabolism such as vitamin D and its nuclear receptor (vitamin D receptor [VDR]) gene polymorphisms may be related to the periodontitis. Along with their role in bone homeostasis, vitamin D and its receptor affect the monocyte phagocytic function and differentiation.[9]

Vitamin D performs its action by interaction with its nuclear receptor (VDR) and form a complex with the gene response element to regulate different biological processes.[10] VDR is a member of a family of transcriptional factors and has a sequence similarity to the receptors of thyroid and steroid hormones.[11]

VDR gene that encodes VDR has nine exons that span approximately 100 kb long located in chromosome 12q13.11, and it is expressed in thyroid gland, kidney, bone, intestine, and other tissues.[12]

Several single nucleotide polymorphisms were identified in the VDR genes like *Taq*I, *Bsm*I, *Apa*I, and *Fok*I, and several studies have examined the relation between these VDR polymorphisms and the susceptibility to periodontitis in different ethnic populations.[13,14]

Hence, the results of studies on VDR gene polymorphism in periodontitis in various ethnicities showed contrary results; we assessed the relation between the VDR genes *Apa*I and *Taq*I polymorphisms and periodontitis in Saudi subjects. To our information from literature, this is the first report about the association of VDR gene *Apa*I G/T and *Taq*I T/C polymorphisms and periodontal disease in Saudi subjects in Makkah region.

Materials and Methods

Study Design and Participants

A total of 172 subjects (86 unrelated patients having moderate-to-severe periodontitis and 86 controls) were enrolled in this cross-sectional study. All participants were Saudi subjects who were chosen from the dental clinic, school of dentistry, Umm AL Qura University, Saudi Arabia. The controls included in the study were free from both periodontal and systemic ailments. Both study groups were matched concerning the age (30–50 years) and gender, and both should have at least 20 teeth. The exclusion criteria were systemic ailments, lactation, pregnancy, preceding orthodontic treatments, immunodeficiency diseases, chemotherapy, and smokers. The size of the sample was considered depending on a preceding study and was increased by approximately 35% to preserve the evaluation at an optimal degree of precision (5%) against the possibility of the sample size reduction due to disavowal and withdrawals.[15] This study got approval from the ethical committee of the School of Dental sciences, Um Al qura University, Kingdom of Saudi. All participating individuals have filled and signed informed consent prior to enrolment in the study.

Clinical Evaluation

The periodontal status of all subjects was evaluated by two trained and calibrated investigators using plaque index (PI), pocket depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP) utilizing Williams periodontal probe.[16-18] Patients with PD ≥5 mm, BOP, CAL ≥3 mm, and bone loss ≥20% on radiographic examination were enrolled in the study. The updated statement of the American Academy of Periodontology was used for characterization of the periodontal disease.[19]

DNA Extraction

Peripheral blood was withdrawn from all individuals in tri-potassium ethylene diamine tetraacetate-coated vacutainer and was utilized for DNA extraction by using commercial kit (Blood Mini Kits, Qiagen, Germany). The purified DNA was used in the polymerase chain reaction (PCR) experiment.

Vitamin D Receptor *ApaI* and *TaqI* Genotyping

A single PCR amplification was performed by using primers that span the *Apa*I and *Taq*I polymorphic sites as described before with slight modifications.[20] The primers used were: 5′-CAGAGCATGGACAGGGAGCAAG-3′ as forward primer and 5′-GCAACTCCTCATGGCT GAGGTCTCA-3′ as reverse primer. After an initial step of 94°C for 5 minutes, 30 cycles of 94°C for 1 minute, 68°C for 1 minute, 72°C for 1 minute, and a final step at 72°C for 7 minutes. The PCR fragment (740 bp) was treated with *Taq*I and *Apa*I restriction enzymes. Fragments were electrophoretically separated on 2% agarose. Digestion with *Apa*I gave single band of 740 bp for the TT genotype and two bands of 530 and 210 bp for the GG genotype. *Taq*I digestion gave two bands of 495 and 245 bp with the TT genotype (due to presence of an obligatory polymorphic site) and three bands of 205, 245, and 290 bp with the CC genotype. The genotypes after restriction digestion are shown in ►**Fig. 1**.

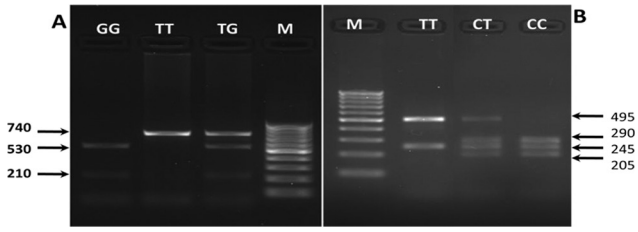

Fig. 1 Agarose electrophoresis of VDR *Apa*I and *Taq*I genotypes. The 740-bp polymerase chain reaction products of the VDR gene were digested with *Apa*I (**A**) and *Taq*I (**B**) restriction enzymes before their resolving in an agarose gel and visualized by UV light. Lane M = DNA molecular ladders (100–1,000 bp). The remaining lanes correspond to the genotypes labeled at the top of each photo. VDR, vitamin D receptor.

Statistics

SPSS version 21 was used for data analysis. Continuous variables were analyzed by utilizing Student's *t*-test, while the categorical data were analyzed by using Chi-square test. A *p*-value <0.05 was considered significant.

Results

Clinical Analysis

The demographic and clinical data are shown in ►**Table 1**. The mean values of PI, CAL, BOP, and PD were higher in the periodontitis group compared with the controls ($p < 0.001$). This confirmed that the two groups were matched adequately.

TaqI Polymorphism

►**Table 2** showed the distribution of genotypes and alleles of *Taq*I polymorphism which was in Hardy–Weinberg equilibrium (HWE) in both groups. In the controls, the TT, TC, and CC genotypes were 41.86, 41.86, and 16.28%, and they were 38.37, 43.02, and 18.61% in the patients' group, respectively. The ratio of T allele was 62.79 and 59.88%, while C allele was 37.21 and 40.12% in controls and the periodontitis subjects, respectively. The *Taq*I genotype and allele frequencies were indifferent between both groups ($p > 0.05$).

ApaI Polymorphism

The genotype and allele distributions of *Apa*I G/T polymorphism of both study groups are presented in ►**Table 2**. The genotype distribution was in HWE in both groups. In the controls, the TT, TG, and GG genotypes were 47.67, 32.56, and 19.77%, and they were 40.69, 44.19, and 15.12% in the periodontal disease, respectively. The percentage of the T allele was 61.63 and 62.79%, while G allele was 38.37 and 37.21% in the controls and the periodontitis subjects, respectively. The *Apa*I genotype and allele frequencies were indifferent between the controls and the periodontitis subjects ($p > 0.05$).

Table 1 Demographic and clinical data of study subjects

Characteristic	Control group (86)	Periodontitis group (86)	*p*-Value
Age (y)	41.84 ± 5.86	42.22 ± 5.96	0.671
Gender (M/F)	51/35	47/39	0.538
BOP (%)	8.53 ± 1.1	49.67 ± 7.06	<0.001
PD (mm)	1.17 ± 0.5	5.06 ± 0.79	<0.001
CAL (mm)	0.67 ± 0.22	4.85 ± 0.88	<0.001
PI (%)	4.88 ± 0.52	48.71 ± 3.66	<0.001

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; PD, pocket depth; PI, plaque index.
Note: Data are shown as mean ± standard deviation.

Table 2 Vitamin D receptor ApaI and TaqI single nucleotide polymorphisms in the study group

	Control ($n = 86$)		Periodontitis ($n = 86$)		*p*-Value	Odds ratio	95% CI
	n	**%**	***n***	**%**			
*Taq*I polymorphism							
Genotypes							
TT	36	41.86	33	38.37		1	
CT	36	41.86	37	43.02	0.74	1.121	0.58–2.166
CC	14	16.28	16	18.61	0.832	0.899	0.384–2.107
Alleles							
T	108	62.79	103	59.88		1	
C	64	37.21	69	40.12	0.841	1.176	0.534–2.588
*Apa*I polymorphism							
Genotypes							
TT	37	47.67	35	40.69		1	
TG	32	32.56	38	44.19	0.508	1.255	0.649–2.427
GG	17	19.77	13	15.12	0.385	1.553	0.656–3.676
Alleles							
T	106	61.63	108	62.79		1	
G	66	38.37	64	37.21	0.547	0.723	0.327–1.598

Note: Chi-square analysis of genotypes between subjects with periodontitis and controls.

Discussion

Periodontal disease is a chronic infection of the tissues surrounding the teeth that leads to destruction of the tooth supporting tissue and alveolar bone owing to the interaction between pathogenic bacteria and host immune response.[1]

The mode of host immune response to the etiologic bacteria depends on genetic factors of the host that may lead to or protect from disease. Several candidate genes were investigated, but the results show controversy. Of these genes is the VDR gene that encodes the VDR that mediates the action of vitamin D in bone metabolism and immunomodulation.[14] We investigated the relationship between VDR *Apa*I and *Taq*I polymorphisms and periodontal disease in a group of Saudi individuals in Makkah region of Saudi Arabia. We found no association between both *Apa*I and *Taq*I polymorphisms and periodontitis; therefore, both VDR *Apa*I and *Taq*I polymorphisms may not carry a risk for periodontitis among Saudis. Similar results were obtained with other investigators in different ethnic populations (Turkish population,[21] Han Chinese subjects,[22] Colombian population,[23] Iranian population,[24] Taiwanese Han ethnic population,[25] the Tamilian population,[26] and the Western Romanian population[27]). However, contrary results were obtained with other investigators. VDR *Apa*I GG genotype was reported to be related to the risk of chronic periodontitis (CP) in the Jordanian population,[28] while *Apa*I T allele was found to be related to the susceptibility to CP in Han Chinese nationality,[29] whereas VDR *Taq*I TT genotype was found to be related to CP in Japanese subjects,[30] Italian population[31] and in CP smokers in Caucasians,[32] while the *Taq*I CC genotype was found to be related to CP in Chinese subjects.[33] These contrary results may be interpreted by different ethnic background or exposure to different environmental conditions.

According to the above, it is obvious that the results of research on the relationship of VDR gene polymorphisms and the risk of periodontal disease differ between various ethnicities.

To our knowledge, this is the first report assessing the relation between VDR *Taq*I and *Apa*I polymorphisms and the risk of periodontal disease in Saudi Arabia.

VDR polymorphisms were studied in other systemic disorders other than periodontal disease such as osteoporosis, diabetes mellitus, cancer, coronary heart disease, and chronic renal failure.[34-38] This study has some limitations, especially the sample size that was not large enough, and so further studies will be required to include a larger cohort of patients with different forms of periodontal disease with clinical data and serological analysis to clarify the role of vitamin D and VDR polymorphism in the progression of periodontitis.

Conclusion

The findings obtained in this study indicated that VDR *Apa*I G/T and *Taq*I T/C polymorphisms might not be related to the risk of periodontal disease in Saudi subjects in Makkah region.

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Conflict of Interest
None declared.

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