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ASSOCIATION BETWEEN VITAMIN D RECEPTOR FOKI POLYMORPHISM AND CHRONIC PERIODONTITIS IN SYRIAN POPULATION

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ABSTRACT: Background: Chronic periodontitis (ChP) is the most common form of periodontal diseases. The progression rate may be influenced by local, systemic conditions and environmental factors that can alter the normal host response to bacterial plaque and affect the susceptibility to disease. Several studies have reported a role for vitamin D receptor (VDR) gene polymorphisms in numerous immunological activities. The present study purpose was to investigate whether FokI polymorphisms in the VDR gene were associated with the incidence of ChP in a Syrian population. **Methods:** The study sample comprised eighty Syrian subjects who were allocated into two groups. 50 patients were included in the group (ChP), whereas 30 matched individuals with no periodontal disease were included in the group (R). DNA was isolated from peripheral blood cells. Subsequently, genotyping of the (VDR) polymorphism was performed by a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis using FokI enzyme. Statistical analysis was carried out to assess the differences between study groups. **Results:** Alleles T and C were present at frequencies of 54% and 46% respectively in group ChP compared to 57% and 43% in group (R). No significant differences were found between the study groups in the frequencies of alleles and genotypes ($p>0.05$). Similarly, no significant association was observed between the severity of the disease and the frequencies of genotypes TT, CT and CC ($p>0.05$). **Conclusion:** VDR FokI polymorphism is not associated with chronic periodontitis in the studied Syrian population.

INTRODUCTION: Periodontitis is a type of chronic inflammatory disease that is initiated by microbial plaque accumulation on the tooth and gingival surfaces at the dentogingival junction [1]. Chronic periodontitis (ChP), the most common form of periodontitis affecting up to 30% of adults [2], is characterized by gingival inflammation, plaque biofilm aggregation (often associated with calculus), attachment loss, slow progressing alveolar bone resorption, as well as periodontal pocket formation and tooth mobility in advanced cases [3, 4].

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Attachment and bone loss is associated with an increase in the proportion of gram-negative bacteria in the subgingival plaque. The mechanisms by which this occurs have not been clearly explained, but these bacteria may have a local effect on the cells of the inflammatory response and the tissue of the host, resulting in a local, site-specific disease process. The outcome of the pathogenic disease process is the result of the mutual interplay between dental plaque and both environmental and genetic factors [5].

Genetic factors may affect the host response to infection and could lead to noticeable changes in its clinical severity. Over the past decade, a strong body of evidence has been gathering regarding the role of gene polymorphisms in susceptibility to periodontal diseases [6].

Some recent studies have implicated the vitamin D receptor (VDR) gene polymorphism as a potential biomarker in assessing the risk of chronic periodontitis [7]. Vitamin D transmits signals to target cells using the vitamin D receptor (VDR), which is composed of both ligand binding and conserved DNA binding domains. The conserved DNA binding domains function primarily as regulators of gene transcription. VDR forms heterodimers with the related retinoid X receptors and binds to DNA to initiate histone modifications, chromatin rebuilding, and RNA polymerase II binding, which is essential for initiation of transcription. The VDR gene is located on chromosome 12q and contains more than 470 single nucleotide polymorphisms (SNPs), some of which modulate 1, 25(OH)2D3 uptake. Therefore, these SNPs can be considered as being proper candidate disease risk variants [8, 9].

The most commonly studied VDR SNPs include rs10735810/rs2228570 (FokI) situated in exon 2. In addition, there are three SNPs in linkage disequilibrium, namely rs1544410 (BsmI) located in intron 8, rs731236 (TaqI) in exon 9 and rs7975232 (ApaI) in intron 9 [10]. The VDR SNP rs2228570 was reported to be associated with severe ChP in a Japanese population. A previous linkage disequilibrium (LD) analysis of SNPs located in the VDR gene demonstrated that rs2228570 was not in LD with any other SNP [2]. Another report found no significant differences in FokI SNP rs#2228570 between ChP patients and controls in Libyan population [5]. Due to the vital dual roles of VDR in the immune response and in bone metabolism, this study aimed to examine the possible association of the SNP FokI in the VDR gene with susceptibility to chronic periodontitis in a Syrian population.

MATERIALS AND METHODS:

Study Population: The study was approved by the Institutional Ethics Committee No. 412, in 14/11/2016 at Damascus University. Patients referred to the Department of Periodontology, Faculty of Dentistry, Damascus University were invited to participate in the study. In total, 80 subjects were enrolled in the study. A thorough medical and dental histories were obtained and a complete periodontal chart was recorded for each participant. Examined periodontal parameters included: plaque index (PI), ginigival index (GI), probing pocket depth (PPD) and clinical attachment loss (CAL). Panoramic dental tomographs were used to confirm the diagnosis.

Participants were assigned into two groups: ChP and R. Group (ChP) comprised 50 patients who were diagnosed with chronic periodontitis according to the guidelines of the American Academy of Periodontology 1999 and fulfilled the following inclusion criteria: patients were ≥40 years of age, have at least 20 teeth, have 2 interproximal sites with CAL ≥ 3 mm and 2 or more interproximal sites with PD ≥4 mm (not on the same tooth) [11].

On the other hand, the control group (R) included 30 individuals who had no periodontal disease and were race and age matching to group ChP. Subjects were excluded if they met any of these conditions: had less than 20 teeth (other than third molars), had any systemic disease that might alter the immunity responses, underwent treatment with antibiotics over the past three months, pregnancy, breastfeeding and smoking.

Finally, all participants gave their written consent after being informed about the nature of the study and its purpose.

Genotype Determination: Phlebotomy procedure was performed, and blood samples were collected using vacutainer tubes. A manual protocol was applied to extract genomic DNA from peripheral blood cells [12]. The extraction solution consisted of urea and proteinase K, lysis buffer (Tris EDTA), washing buffer (10 mM NaCl/10 mM EDTA), SDS 20% and NaCl 5M. The genomic DNA was quantified by absorbance at 260 nm using a spectrophotometer, and then stored at -20 °C until future use.

Subsequently, the polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) technique was utilized for genotyping the VDR polymorphism. Primers used had the following sequences: 5'-AGCTGGCCCTGGCACT GACTCTGGCTCT-3' (forward), 5'-ATGGAAAC ACCTTGCTTCTTCTCCCTC-3' (reverse) [13]. About 20 pM of each forward and reverse primers were added to a reaction mixture containing: 45 µl of PCR SuperMix 1.1 (1unit of Taq DNA

polymerase, 22 mM Tris-HCl (pH 8.4), 55mM KCl, 1.65 mM Mg Cl_2, 0.2 mM of each $dNTP_s$), template DNA 1 µl (150-250 ng), and nuclease-free water to reach a final volume of 50 µl. The PCR program was performed for 30 cycles and consisted of: an initial denaturation for 5 min at 94.5 °C, 30 sec at 94 °C for denaturation, 60 °C for 30 sec for annealing, and 72°C for 30 sec for elongation, followed by a final extension for 10 min at 72 °C [13]. Later, Agarose gel electrophoresis was used to monitor the PCR products. Finally, Individuals were scored as FF (TT) homozygotes, Ff (CT) heterozygotes, or ff (CC) homozygotes according to the digestion pattern by *FokI* fast digest enzyme.

Statistical Analysis: The collected data was analyzing using SPSS software (version 22, IBM Corp, NY, USA). The Hardy-Weinberg equilibrium was verified. Mean values and standard deviations (SD) were calculated for all descriptive variables. The Chi-square test was used to assess the differences in alleles and genotypes frequencies among study groups. The odds ratio (OR) and 95% confidence intervals (CI) were also calculated and all analyses were considered statistically significant at the level of $p<0.05$.

RESULTS: Table 1 shows the demographic parameters of the study groups. In this study, a total of 80 subjects between 40 and 70 years of age were recruited. ChP group comprised 50 patients with 17 (34%) females and 33 (66%) males, whereas subjects in the control group (R) were 12 (40%) females and 18 (60%) males. In group ChP the mean age of patients was (64 ± 0.722) compared to (50 ± 0.63) in the control group (R). Participants were further divided about their age group into three groups: 40-49 years, 50-59 years and 60-69 years. Moreover, patients in the ChP group were classified into three subgroups based upon the periodontal pocket depth measurements as follows: mild (PD=4 - 5mm), moderate (PD= 6-7mm) and severe (PD>7mm).

TABLE 1: DEMOGRAPHIC PARAMETERS OF THE STUDY POPULATION

Patient data	Control (R)	Chronic periodontitis (ChP)			
		Mild	Moderate	Severe	total
N	30	11	15	24	50
		Sex n (%)			
Male	18 (60%)	8	10	15	33 (66%)
Female	12 (40%)	3	5	9	17 (34%)
Age (years) mean ± SD	50 ± 0.63				64 ± 0.722
		Age group n (%)			
40-49	17(56.67%)	2	12	10	24 (48%)
50-59	11(36.67%)	7	3	11	21 (42%)
60-69	2(6.66%)	2	0	3	5 (10%)

Hardy-Weinberg Equilibrium (HWE) was verified. All population data demonstrated the goodness of fit with HWE as there were no differences between the observed and expected frequencies of genotypes and alleles in both study groups ($p>0.05$) **Table 2**.

TABLE 2: FREQUENCIES OF EXPECTED AND OBSERVED GENOTYPES ACCORDING TO HARDY-WEINBERG EQUILIBRIUM

	Genotypes	Expected Genotypes	Observed Genotypes	P-value
Controls	TT	9.645	11	0.199
	CT	14.73	12	
	CC	5.625	7	
Patients	TT	14.58	15	
	CT	24.84	24	0.2
	CC	10.58	11	

The data from **Table 3** show that allele T was detected in 54% of patients in group ChP compared to 57% in group (R). Similarly, allele C had a frequency of 46% in group ChP compared to 43% in the control group. No statistical differences were observed in allele frequencies between study groups ($p>0.05$). Likewise, in the ChP group, genotype TT, CT and CC had the following frequencies 30%, 48%, and 22% respectively.

The corresponding frequencies in the group (R) were 37%, 40%, and 23% respectively. There were no statistically significant differences in the frequencies of genotypes among both study groups (p>0.05).

TABLE 3: THE GENOTYPES AND ALLELES FREQUENCIES

	Control (n = 30)	Patient (n = 50)	P*	OR	CI (95%)
Genotypes					
TT	11 (36.7%)	15 (30%)	0.538	0.740	0.284 - 1.929
CT	12 (40%)	24 (48%)	0.486	1.385	0.553 - 3.465
CC	7 (23.3%)	11 (22%)	0.890	0.927	0.315 - 2.725
Alleles					
T	34 (56.7%)	54 (54%)	0.890	1.079	0.367 - 3.173
C	26 (43.3%)	46 (46%)	0.418	1.489	0.567 - 3.910

(*) P = Chi square, OR = Odd Ratio, CI = Confidence intervals

As can be seen from **Table 4** analysis of the distribution of alleles in the ChP group according to disease severity revealed no significant differences in the detected frequencies of allele T and C among the three groups: mild, moderate and severe (p>0.05). Similarly, no statistically significant differences were recorded in the frequencies of genotypes concerning disease severity (p>0.05).

TABLE 4: THE FREQUENCIES OF ALLELES AND GENOTYPES IN GROUP CHP ACCORDING TO DISEASE SEVERITY

Genotypes	Mild	Moderate	Severe	*P**
TT	4 (26.67%)	5 (33.33%)	6 (40%)	0.749
CT	4 (16.67%)	6 (25%)	14 (58.33%)	0.366
CC	3 (27.27%)	4 (36.36%)	4 (36.36%)	0.682
Alleles				
T	12 (22.22%)	16 (29.63%)	26 (48.15%)	0.682
C	10 (21.8%)	14 (30.4%)	22 (47.8%)	0.778

DISCUSSION: In the present study, 80 Syrian subjects (50 diagnosed with ChP and 30 matched controls) were recruited. An attempt was undertaken to analyze the vitamin D receptor gene polymorphisms and to assess any possible association between ChP susceptibility and FokI VDR SNP. No significant differences were found between the study groups in the frequencies of alleles and genotypes at FokI position. The findings of this study are in line with many other recent studies that also failed to find any correlation between FokI SNP and ChP as El Jilani *et al*., [5] reported no significant differences in FokI SNP rs#2228570 between ChP patients and controls in Libyan population and Wang *et al*., on Asians [2].

The results of the present study were in disagreement with data obtained from the study of Naito *et al*., 2007 who showed that FokI VDR polymorphism was less likely to develop severe chronic periodontitis than non-F-carriers in a Japanese population. The haplotype analysis of the three combined VDR polymorphisms (ApaI, BsmI and FokI) revealed that the Abf homozygote had a notably higher prevalence of severe chronic periodontitis [14]. The reason for this discrepancy can be due to the ethnic differences between the studied populations and other differences in sample size and age groups. Smoking has been identified as a major environmental risk factor associated with increased incidence and severity of periodontitis [15]. Thus, smokers were excluded from our study to have less confounding factors when analyzing any genetic association with periodontitis. Our study had a higher percentage of men with ChP but without any statistically significant difference. The causes of these sex differences have not been explained clearly. However, it could be suggested that men have poorer oral hygiene, less positive thinking toward oral health and dental visit interaction. While, women, on the other hand, adopt a more positive attitude towards their oral health and have a better awareness of their dental needs.

VDR FokI polymorphism restriction site is located in exon 2 in the 5′ coding region of the gene. This polymorphism results in different translation initiation sites on VDR. Thymine (T) to a cytosine (C) conversion in the first translation initiation

codon ATG (methionine) generates long and short variants of VDR [16]. Nonetheless, the shorter VDR exhibits higher transcriptional activity [16] and transmits signals related to bone resorption and inflammation more efficiently compared with the normal protein, which may impact the susceptibility to ChP [2]. To the best of our knowledge, this report is the first study to examine the association between VDR gene polymorphisms and susceptibility to ChP in Syria. The findings of this research can be explained in light of the etiopathogenic of periodontal disease. Chronic Periodontitis (ChP) is one of the most common inflammatory diseases affecting the tooth-supporting tissues. It is caused by microorganisms that produce dental plaque biofilm that can trigger the initiation of the inflammation and induce immune responses. ChP destroys soft tissues and the alveolar bone that supports the teeth leading eventually to tooth loss. Bacteria, immune activities, environmental and genetic interactions can be considered major risk factors in the etiology of ChP [7].

Periodontitis is a complex multifactorial disease that is affected by the interaction between multiple genes and environmental factors. Such complex genetic diseases are associated with changes in some SNPs that can eventually have a small contribution to the increased risk of periodontal disease. Hence, SNPs are considered to be disease modifiers in periodontal disease [17]. It was estimated in a recent study that there is at least 20 known disease-modifying genes associated with periodontal disease [18]. Additionally, the number and type of functional genes may differ in different ethnic groups [19].

Results of this study suggested that examining only one SNP in one position for a particular gene (FokI VDR) was not enough to establish an association with ChP in the studied population. This finding can be attributed to the properties of VDR which regulates the expression of numerous genes involved in calcium/phosphate homeostasis, cellular proliferation and differentiation, and immune response, largely in a ligand-dependent manner, so any SNP may alter the amount of protein expression or protein size and consequently the expression of other genes. On a parallel note, it could be of greater importance to assess the effects of the VDR gene polymorphism haplotype on susceptibility to ChP. Further studies should extensively analyze gene-gene and gene-environment interactions using different ethnic groups and a larger number of samples to obtain a better insight into the pathogenesis of ChP. It is worth stating that this study had its shortcomings. The investigated sample size was somewhat small, and the gene polymorphism analysis was performed only the FokI position in the VDR gene.

CONCLUSION: Within the limits of this study it can be concluded that FokI VDR polymorphism is not associated with chronic periodontitis in the studied Syrian population. Nevertheless, further studies need to be conducted to evaluate other SNPs in the VDR gene in a larger sample size.

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CONFLICT OF INTEREST: The authors declare that this study has no conflict of interest.

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