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ORIGINAL ARTICLE

SINGLE NUCLEOTIDE POLYMORPHISMS IN IL-1A RS1800587, IL-1B RS1143634 AND VITAMIN D RECEPTOR RS731236 IN STAGE III GRADE B/C PERIODONTITIS

Özturk Özener H.[1], Tacal Aslan B.[2], Eken B.F.[2], Agrali Ö.B.[1], Yildrim H.S.[1], Altunok E.Ç.[3], Ulucan K.[2], Kuru L.[1]

*Corresponding Author: Assist. Prof. Dr. Hafize Öztürk Özener, Address: Marmara University, Faculty of Dentistry, Department of Periodontology Basıbuyuk, Maltepe, Istanbul, Turkey. Fax number: +90 216 421 02 21; E-mail: hafize.ozturk@marmara.edu.tr

ABSTRACT

The purpose of the study is to determine the prevalence of interleukin (IL)-1A (rs1800587), *IL-1B* (rs1143634) and vitamin D receptor (*VDR*) (*TaqI,* rs731236) gene polymorphisms in the Turkish population and their association with Stage III Grade B/C periodontitis. Systemically and periodontally healthy individuals (N = 100) and Stage III Grade B/C periodontitis patients (N=100) based on clinical and radiographic examination were included in this research. Clinical attachment level, probing depth, bleeding on probing, plaque and gingival indices of the subjects were measured. Genotyping of *IL-1A* (rs1800587), *IL-1B* (rs1143634) and *VDR* (rs731236) polymorphisms was conducted by Real Time PCR. Allelic and genotypic distributions of *IL-1A* (rs1800587) gene polymorphism were not associated with periodontitis (p>0.05). In *IL-1B* (rs1143634) gene polymorphism, the C allele was detected more frequently in healthy individuals compared with the periodontitis patients (p=0.045). CC genotype and C allele in *VDR* (rs731236) gene polymorphism was higher in periodontitis patients (p=0.031, p=0.034, respectively). In comparison with Grade B periodontitis patients and healthy subjects, CC genotype and C allele were observed more frequently in the Grade B periodontitis in terms of alleles (C/T) and genotypes for *VDR* (rs731236) polymorphism (p=0.024, p=0.008, respectively). This study presents that the *VDR* (rs731236) polymorphism are associated with enhanced susceptibility to Stage III periodontitis in the Turkish population. Furthermore, *VDR* (rs731236) polymorphism may be used as an identification criteria to discriminate Grade B and Grade C in Stage III periodontitis.

Keywords: Interleukin-1alpha, Interleukin-1beta, Vitamin D receptor, Periodontitis, Polymorphism

INTRODUCTION

As a chronic multifactorial infectious disease, periodontitis has been defined as one of the main public health problems with its high prevalence and it has been shown to lead to disability and tooth loss, impairment on aesthetic and chewing function, a presumed negative effect on general health, social inequality, and impaired quality of life [1]. It is described as microbially originated, host mediated inflammation resulting in periodontal attachment loss and, ultimately, tooth loss [2]. The etiopathogenetic mechanisms of periodontitis have not been completely discovered. Although the primary etiology of the disease is the pathogenic anaerobic bacteria found in the subgingival dental plaque; it has been shown that host susceptibility and the genetic background of individuals play substantial roles in the onset and development of the disease, in particular, with a subset of genes predominantly believed to be part of the pathological processes [3]. Researchers have completed studies on genetic association regarding the polymorphism analysis to further clarify the role of each of these genes. Polymorphisms may play a part in the presentation and/or outcome of diseases. This can also be done through conferring a degree of risk or protection from the disease.

Gene polymorphism, namely interleukin (IL)-1, is the most pronounced gene polymorphism studied in patients with periodontal diseases [4]. *IL-1A* and *IL-1B*, as mem-

[1] Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey.

[2] Department of Basic Medical Sciences Medical Biology and Genetics, Faculty of Dentistry, Marmara University, Istanbul, Turkey.

[3] Department of Biostatistics and Medical Informatics, Faculty of Medicine, Yeditepe University, Istanbul, Turkey.

bers of IL-1 family, are the proinflammatory cytokines that are expressed in response to cell damage and impact cell differentiation, proliferation, and apoptosis [5]. Particularly, single nucleotide polymorphism (SNP) in position *IL-1A* −889 and in position *IL-1B* +3953 (also known as +3954) are the most investigated SNPs. In 1997, Kornman et al. [6] reported on the simultaneous presence of both *IL-1A* −899 and *IL-1B* +3953 minor alleles, known as "composite genotype", and an elevated severity of chronic periodontitis in a subset of non-smoker Caucasian subjects. According to the findings of a meta-analysis, comprised of 27 studies published from 1997 to 2008, 19 of these studies found a correlation between the carriage of minor IL-1 alleles and periodontitis [7]. Controversial findings have also been reported in studies where no association has been found between rs1143634 and rs1800587 gene polymorphisms and chronic and/or aggressive periodontitis [8-12]. Da Silva et al. [13] reported a meta-analysis from 21 case-control research studies, published between 1998-2015, and uncovered an association between periodontitis and rs1800587. Another meta-analysis of 54 case/control studies, containing 9376 subjects, showed that rs1143634 polymorphism was linked to a high risk of chronic periodontitis in both the overall analysis and different ethnicities, such as Asian and Caucasian [14].

The vitamin D receptor (*VDR*) is present in many cells of the immune system, such as in monocytes and dendritic cells, as well as in bone cells such as osteoblasts [15] and osteoclasts. Beyond its established function in bone homeostasis, vitamin D also has significant anti-inflammatory and immunomodulatory properties [16]. Genetic polymorphisms in the *VDR* gene have been shown to be linked to bone mineral density and also bone-associated diseases [17,18]. Results of studies examining the association between periodontitis and *VDR* gene polymorphisms at the *Apa*I*, Bsm*I, *Fok*I, and *Taq*I restriction sites or their combinations are somewhat controversial [19, 20], and it is not clear which of the *VDR* polymorphisms are effective in periodontal disease susceptibility. A meta-analysis involving 15 studies, reported that *VDR Taq*I, *Apa*I and *Bsm*I gene polymorphisms were associated with chronic periodontitis in Asians, yet not in Caucasians, whereas there was no relationship between chronic periodontitis and *Fok*I polymorphism [21]. However, another meta-analysis of 18 studies concluded that chronic periodontitis linked to only *Taq*I polymorphism in Asians [22]. A more recent meta-analysis concluded that no significant associations were present between *Taq*I polymorphism and chronic periodontitis in Caucasian, Asian, African and mixed populations [23].

Until now, the effects of the genetic factor on different forms of periodontal disease have been investigated in several studies, whereas the results obtained by different researchers vary. This study hypothesized that *IL-1A* (rs1800587), *IL-1B* (rs1143634) and *VDR* (rs731236) gene polymorphisms may confer susceptibility to periodontitis. Thus, the purpose of the present study was to determine the frequencies of *IL-1A* (rs1800587), *IL-1B* (rs1143634) and *VDR* (*Taq*I, rs731236) gene polymorphisms in Stage III Grade B/C periodontitis patients in the Turkish population.

METHODS

Study Population

The protocol of this cross-sectional study was approved by the Clinical Studies Ethical Committee of Faculty of Dentistry, Marmara University on 23.10.2017 with the reference number 2017-143, and registered at clinicaltrials.gov (identification number NCT04806971). According to the 2013 revision of the 1975 Helsinki Declaration, each participant signed an informed consent form prior to the study. All individuals provided a written informed consent. The present study recruited 200 individuals from the Department of Periodontology, Faculty of Dentistry, Marmara University between January 2018 and September 2019. The study population was made up of 100 periodontitis patients and 100 periodontally healthy subjects of Turkish origin.

Inclusion criteria for entry were as follows: previously untreated periodontitis patients, systemically healthy study population. Exclusion criteria included smoking (current and past); presence of systemic disease such as diabetes, rheumatic fever, kidney or liver diseases, neurologic deficiencies, immunologic diseases; pregnancy; received antibiotics within the last 6 months; use of any medication that may influence periodontium (chronic use of non-steroidal anti-inflammatory drugs, cyclosporine, nifedipine, phenytoin, etc.).

Periodontitis group: Stage III, Grade B/C periodontitis patients were diagnosed on the base of the new classification criteria [2,24]. Stage III periodontitis patients enrolled had ≥20 teeth, probing depth (PD) ≥6 mm, clinical attachment loss ≥5 mm, and bone loss reaching to the middle third of the root radiographically. Grade B was assessed by indirect consideration of radiographic bone loss throughout the most affected tooth in the dentition as a function of age (% bone loss/age: 0.25-1.0), and Grade C was evaluated based on the radiographic bone loss in the most affected tooth in whole dentition as a function of age (% bone loss/age: >1.0). Subjects in the periodontitis group were categorized into two subgroups, 51 patients Grade B and 49 patients Grade C.

Healthy group: The periodontally healthy subjects with the absence of any sign of clinical inflammation, not

having periodontitis history; absence of detectable bone and/or attachment loss; <10% of sites with bleeding on probing (BOP); PD ≤3 mm; presence of 28 permanent teeth without extensive restorations or caries. In addition, all subjects in the healthy group did not show any local or systemic pathology.

Periodontal Examination

Periodontal examinations were carried out by the same periodontist (HOO) using a periodontal probe (PCP 15 UNC, Hu-Friedy, Chicago, USA). Plaque index (PI) [25] and gingival index (GI) [26] were evaluated at 4 sites, PD, clinical attachment level (CAL) and BOP at 6 sites per tooth, excluding third molars. A calibration session was performed by using 10 subjects (not part of the study groups) to assess the intra-examiner reliability and full mouth PD and CAL scores were measured at two separate sessions, 24 hours apart. The intra-examiner correlation for PD and CAL measurements were calculated as 92.2% and 93% reproducibility, respectively.

Genotyping

4–6 ml venous blood samples were collected from the antecubital fossa of each individual and stored in ethylenediaminetetraacetic acid (EDTA) vacationer. DNA was extracted from the blood by using commercially available kits (Invitrogen, USA), following manufacturer's guidelines. Sections of the *IL-1A*, *IL-1B* and *VDR* genes that contain the SNPs of interest were amplified. All genotyping assays were validated prior to processing the study samples. After the validation step, negative controls (water), positive controls of known genotypes and a subset of study samples were genotyped in duplicate. All samples were genotyped for rs1800587, rs1143634 and rs731236 polymorphisms using Real-Time PCR (Thermofisher Quantstudio 3, USA) by using a Taqman genotyping assay (Catalog no: 4362691 Thermofisher, USA). The 10 µl reaction volume comprised of 5 µl genotyping master mix (Applied Biosystems, Foster City, CA), 0.5 µl genotyping assay (Applied Biosystems), 3.5 µl nuclease-free H_2O (Thermofisher, USA), and 1 µl DNA. Reactions were incubated in a 96-well optical plate for denaturation at 92°C for 15 s, followed by annealing and extension at 60°C for 1 min (40 cycles).

Statistical Analysis

SPSS software version 25 was used for analyses. The descriptive statistics were presented using mean and standard deviation. Frequencies and percentages were used for categorical data. The Kolmogorov Smirnov test was performed to investigate to whether the variables are normally distributed or not. For association analysis between SNPs and periodontitis susceptibility, various genetic models were used by SNPassoc. The Mann-Whitney U test was done to compare the groups, since the variables were not normally distributed. Chi-Square test and Fisher's exact test, where appropriate, was applied for comparison of the proportions of the groups. The study population obeys the rule of the Hardy-Weinberg equilibrium law, which was carried out with a goodness-of-fit $\chi 2$. Odds ratios with a 95% confidence interval were calculated for risk factors. A 5% type-I error level was used to interpret a statistical significance.

RESULTS

Table 1 demonstrates demographic data and clinical features of the periodontitis patients and the healthy subjects. All periodontal clinical parameters in the patients with periodontitis were significantly higher comparison to the healthy individuals, as expected ($p<0.000$).

In Table 2, the distributions of polymorphisms in all study populations agreed to the Hardy–Weinberg equilibrium ($p > 0.05$), except *IL-1B* (rs1143634) in the healthy group ($X^2=7.75$; $p < 0.05$). Comparisons of genotype frequencies between the periodontitis patients and healthy individuals are presented in Table 2. *IL-1A* (rs1800587) and *IL-1B* (rs1143634) polymorphisms did not show any association with periodontitis (OR=1.142, p=0.793, OR=1.726, p=0.371, respectively). Considering the *VDR TaqI* gene polymorphism, the frequencies of TT genotype in the periodontitis and healthy subjects were 36% and 49%, respectively, and CC genotype 19% and 10%, respectively. The results showed that the genotype CC was associated with a 2.586 times higher risk of periodontitis with a statistically significant risk coefficient (p=0.031).

Table 1. Periodontal clinical parameters in study population

Parameters	Periodontitis N=100 Mean±SD	Healthy N=100 Mean±SD	P value
Age	39.61±8.74	29.50±4.98	0.000*
Range (years)	(22-59)	(21-55)	
Gender (%)			
Male	43 (43)	38 (38)	0.565†
Female	57 (57)	62 (62)	
PD (mm)	2.88±0.79	1.79±0.33	0.000*
CAL (mm)	3.14±0.88	1.81±1.79	0.000*
PI	1.70±0.45	0.34±0.20	0.000*
GI	1.56±0.41	0.26±0.23	0.000*
BOP (%)	58.82±21.91	8.77±6.25	0.000*

*Mann Whitney U test, †Chi-Square, p<0.05.
PD: Probing Depth, CAL: Clinical Attachment Level, PI: Plaque Index, GI: Gingival Index, BOP: Bleeding on Probing.

Table 2. Genotypic frequency of IL-1A, IL-1B and VDR polymorphisms in periodontitis and healthy population

Gene SNPs (rs number)	Genotype	Periodontitis N= 100 N (%)	Healthy N=100 N (%)	χ2 P value	OR (95% CI)	Fisher exact P value
IL-1A^{-889} (rs1800587)	**CC**	49 (49)	56 (56)	0.793	1.142(0.420-3.107)	0.804
	CT	42 (42)	35 (35)	0.727	0.833(2.327-2.327)	0.796
	TT	9 (9)	9 (9)			
	HWE	1.000	0.309			
IL-1B^{+3954} (rs1143634)	**CC**	60 (60)	74 (74)	0.371	1.726 (0.52-5.716)	1.000
	CT	33 (33)	21 (21)	0.858	0.890 0.249-3.176)	0.333
	TT	7 (7)	5 (5)			
	HWE	0.867	0.005			
VDR *Taq*I (rs731236)	**CC**	19 (19)	10 (10)	0.031	2.586(1,074-6.224)	0.034
	CT	45 (45)	41 (41)	0.192	1.494 (0.817-2.732)	0.222
	TT	36 (36)	49 (49)			
	HWE	0.464	0.742			

OR: odds ratio value, CI: confidence intervals, HWE: Hardy-Weinberg equilibrium, p<0.05.

Table 3. Allelic frequency of IL-1A, IL-1B and VDR polymorphisms in periodontitis and healthy population

Gene SNPs (rs number)	Allele	Periodontitis N= 100 N (%)	Healthy N=100 N (%)	χ2 P value	OR (95% CI)	Fisher exact P value
IL1A^{-889} (rs1800587)	C	140 (70)	147 (73.5)	0.437	1.188 (0.768-1.838)	0.505
	T	60 (30)	53 (26.5)			
IL1B^{+3954} (rs1143634)	C	153 (76.5)	169 (84.5)	0.045	1.167 (1.012-2.770)	0.058
	T	47 (23.5)	31 (15.5)			
VDR *Taq*I (rs731236)	C	83 (41.5)	61 (30.5)	0.022	1.61 (1.071-2.441)	0.029
	T	117 (58.5)	139 (69.5)			

OR: odds ratio value, CI: confidence intervals, p<0.05.

The allelic distributions of each gene polymorphisms are presented in Table 3. Allele frequency regarding *IL-1A* (rs1800587) demonstrated no significant differences between the healthy individuals and the periodontitis patients. The C allele in *IL-1B* (rs1143634) was detected more frequently in healthy individuals than patients (84.5% versus 76.5% OR=1.167, p=0.045). In *VDR Taq*I gene polymorphism, the C allele frequency in the periodontitis patients (83%) was higher than in the healthy subjects (61%). The C allele was statistically significant in the periodontitis patients with an OR of 1.61 (95% CI= 1.071–2.441, p=0.022).

Model wise genotypic distributions regarding codominant, dominant, recessive and overdominant models are presented in Table 4. Model wise genotypic distribution of *IL-1A* (rs1800587) did not demonstrate any association with periodontitis (Table 4). Regarding the *IL-1B* (rs1143634) polymorphic region, the frequency of CC genotype was higher in healthy subjects and associated with a decreased risk of periodontitis under dominant model [OR (95% CI) =0.527 (0.289-0.960), p=0.035]. Likewise, *VDR Taq*I gene polymorphism showed alliance with periodontitis, on the other hand, the dominant, recessive and overdominant models did not demonstrate any relation with periodontitis (Table 4).

Associations between different grades of periodontitis and gene polymorphisms in *IL-1A* (rs1800587), *IL-1B* (rs1143634) and *VDR TaqI* are demonstrated in Table 5. Both allele and genotype frequency of *IL-1A* (rs1800587) gene polymorphism did not show any association with any grade of periodontitis (p>0.05). The C and T alleles frequencies of *IL-1B* (rs1143634) polymorphism were 76 (74.5%) and 26 (25.5%) in the Grade B periodontitis, 77 (78.6%) and 21 (21.4%) in the Grade C periodontitis and 169 (84.5%) and 31 (15.5%) in the healthy subjects, respectively. The difference was found to be statistically significant only between the Grade B periodontitis and healthy subjects [OR=1.865; 95% CI (1.037-3.355), p=0.036]. A comparison of the *VDR Taq*I genotypes and alleles between the Grade B periodontitis and healthy subjects revealed that the TT genotype, and T allele were more frequently observed in the healthy subjects (TT *vs*. CC, TT vs. CT, T *vs*. C, p<0.05). In the evaluation of *Taq*I polymorphism in Grade B and C periodontitis, individuals with the CT genotype have a 3.207 times higher risk of Grade B periodontitis than individuals with the TT genotype. [CT *vs*. TT, OR (95% CI) =3.207 (1.286-7.997), p=0.011].

Table 4. Model wise genotypic distribution of IL-1A, IL-1B and VDR in periodontitis and healthy population

Genotypes and alleles	Periodontitis N (%)	Healthy N (%)	OR (95% CI)	χ2 P value
IL1A^{-889} (rs1800587)				
C	140 (70)	147 (73.5)	1.188 (0.768-1.838)	0.437
T ref	60 (30)	53 (26.5)		
Codominant model				
CC	49 (49)	56 (56)	1.142 (0.420-3.107)	0.793
CT	42 (42)	35 (35)	0.833 (2.327-2.327)	0.727
TT ref	9 (9)	9 (9)		
Dominant model				
CC ref	49	56		
CT+TT	51	44	0.754 (0.432-1.316)	0.322
Recessive model				
CC+CT ref	91	91		
TT	9	9	1.000 (0.380-2.634)	1.000
Overdominant model				
CC+TT ref	58	65		
CT	42	35	0.744 (0.420-1.317)	0.309
IL1B^{+3954} (rs1143634)				
C	153 (76.5)	169 (84.5)	1.167 (1.012-2.770)	0.045
T ref	47 (23.5)	31 (15.5)		
Codominant model				
CC	60 (60)	74 (74)	1.726 (0.52-5.716)	0.371
CT	33 (33)	21 (21)	0.890 (0.249-3.176)	0.858
TT ref	7 (7)	5 (5)		
Dominant model				
CC ref	60 (60)	74 (74)		
CT+TT	40 (40)	26 (26)	0.527 (0.289-0.960)	0.035
Recessive model				
CC+CT ref	93 (93)	95 (95)		
TT	7 (7)	5 (5)	0.699 (0.214-2.282)	0.522
Overdominant model				
CC+TT ref	67 (67)	79 (79)	0.5397 (0,2855-1.021)	0.058
CT	33 (33)	21 (21)		
VDR *Taq*I (rs731236)				
T	117 (58.5)	139 (69.5)	1.616 (1.07-2.44)	0.022
C ref	83 (41.5)	61 (30.5)		
Codominant model				
TT	36 (36)	49 (49)	2.586 (1.074-6.224)	0.034
CT	45 (45)	41 (41)	1.73 (0.721-4.152)	0.218
CC ref	19 (19)	10 (10)		
Dominant model				
TT ref	36 (36)	49 (49)		
CT+CC	64 (64)	51 (51)	0.585 (0.332-1.031)	0.063
Recessive model				
TT+CT ref	81 (81)	90 (90)		
CC	19 (19)	10 (10)	0.474 (0.208-1.078)	0.075
Overdominant model				
TT+CC ref	55 (55)	59 (59)		
CT	45 (45)	41 (41)	0.845 (0.485-1.487)	0.568

OR: odds ratio value, CI: confidence intervals, $p<0.05$.

Table 5. Genotypic and allelic frequency of IL-1A, IL-1B and VDR polymorphisms in Grade B and C periodontitis and healthy population

Gene SNPs (rs number)	Genotypes and Alleles	Grade B Periodontitis N= 51 N (%)	Grade C Periodontitis N= 49 N (%)	Healthy N= 100 N (%)	Grade B Periodontitis *vs* Healthy			Grade C Periodontitis *vs* Healthy			Grade B Periodontitis vs Grade C Periodontitis		
					$\chi 2$ (p value)	OR (95% CI)	Fisher exact (p value)	$\chi 2$ (p value)	OR (95% CI)	Fisher exact (p value)	$\chi 2$ (p value)	OR (95% CI)	Fisher exact (p value)
IL1A^{-889} (rs1800587)	**CC**	26 (51)	23 (46.9)	56 (56)	0.608	0.718 (0.179-2.874)	0.749	0.403	1.623 (0.518-5.082)	0.543	0.277	2.261 (0.507-10.07)	0.470
						0.539 (0.129-2.175)			1.167 (0.362-3.756)			2.200(0.485-9.983)	
	CT	22 (43.1)	20 (40.8)	35 (35)	0.323		0.515	0.796		1.000	0.300		0.485
	TT	3 (5.9)	6 (12.2)	9 (9)									
	C allele	74 (72.5)	66 (67.3)	147 (73.5)	0.806	1.07 (0.625-1.831)	0.890	0.241	1.372 (0.809-2.323)	0.273	0.422	1.281 (0.699-2.349)	0.444
	T allele	28 (27.5)	32 (32,7)	53 (26.5)									
IL1B^{+3954} (rs1143634)	**CC**	29 (56.9)	31 (63.3)	74 (74)	0.304	0.490 (0.123-1.953)	0.445	0.635	0.698 (0.157-3.103)	0.695	0.659	0.702 (0.144-3.407)	0.709
						1.071 (0.249-4.603)			1.190 (0.246-5.794)			0.900(0.173-4.669)	
	CT	18 (35.3)	15 (30.6)	21 (21)	0.926		1.000	0.828		1.000	0.900		1.000
	TT	4 (7.8)	3 (6.1)	5 (5)									
	C allele	76 (74.5)	77 (78.6)	169 (84.5)	0.036	1.865 (1.037-3.355)	0.043	0.205	0.673 (0.363-1.245)	0.255	0.498	0.797 (0.413-1.537)	0.510
	T allele	26 (25.5)	21 (21.4)	31 (15.5)									
VDR *Taq*I (rs731236)	**CC**	9 (17.6)	10 (20.4)	10 (10)	0.024	3.392 (1.142-10.075)	0.038	0.136	3.769 (1.295-10.975)	0.188	0.564	1.592 (0,515-4,922)	0.300
									0.831 (0.388-1.780)			3.207 (1.286-7.997)	
	CT	29 (56.9)	16 (32.7)	41 (41)	0.012	2.666 (1.229-5.785)	0.015	0.634		0.702	0.011		0.014
	TT	13 (25.5)	23 (46.9)	49 (49)									
	C allele	47 (46.1)	36 (36.7)	61 (30.5)	0.008	1.947 (1.190-3.185)	0.011	0.281	1.323 (0.795-2.202)	0.295	0.180	1.472 (0.836-2.592)	0.198
	T allele	55 (53.9)	62 (63.3)	139 (69.5)									

OR: odds ratio value, CI: confidence intervals, $p<0.05$.

DISCUSSION

Recent evidence has shown that, with the continuous advancement of genetic engineering technology, gene polymorphism can be an important basis for the individual differences in the development and progression of periodontitis. In this study, the frequencies of *IL-1A* (rs1800587), *IL-1B* (rs1143634) and *VDR* (rs731236) gene polymorphisms were evaluated for the first time in Stage III Grade B/C periodontitis patients in comparison with periodontally healthy individuals. Among different genotyping methods, Real-time PCR that could rapidly and simultaneously detect SNPs was preferred in this study.

SNPs from the promoter region, which play an essential role the transcriptional regulation in the coding region, can affect the expression of a gene. The genes in IL-1 family possess allele polymorphisms in which SNP -889C/T (rs1800587) in *IL-1A* gene and SNP +3654 (rs1143634) in *IL-1B* gene are researched extensively. Our findings revealed that the genotypic distributions of *IL-1A* (rs1800587) and *IL-1B* (rs1143634) gene polymorphisms are not associated with Stage III periodontitis. These results are consistent with the previous studies reporting no relationship between SNPs rs1800587 or rs1143634 and neither chronic nor aggressive periodontitis [8,27-33]. On the other hand, contradictory findings were published declaring significant associations between either of the two SNPs and periodontal diseases [29, 34-39]. Majumder et al. [40] and Wagner et al. [41] concluded that *IL-1A* (rs1800587) polymorphism was related to the susceptibility of chronic periodontitis. Recently, a meta-analysis of ethnicity assessment revealed that *IL-1A* (rs1800587) gene polymorphism is associated with an increased risk of chronic periodontitis in Europeans, US Americans and Africans, but not in Algerians and Mexicans [42]. The results of the present study displayed T allele for *IL-1B* (rs1143634) to be associated with the periodontitis patients, while the C allele was associated with the healthy individuals. Similarly, T allele in *IL-1B* (+3954C/T) was linked to high risk of periodontal disease in Asians, Caucasians, and in mixed populations, however not in Africans [14]. Considering that these differences may be related to the population, the literature contains no data on the Turkish population regarding *IL-1A* (-889C/T) and only 2 research studies have been published about *IL-1B* (+3954C/T). Yücel et al. [43] stated that the distribution of allele and genotype frequencies for *IL-1B* (+3954C/T) were similar among chronic periodontitis, aggressive periodontitis and healthy control groups. On the other hand, our result was contrary to the results of Güzeldemir et al. [37] who show that susceptibility to localized aggressive periodontitis is increased by homozygosity for allele 1 of rs1143634 which referred to (C→T) at position +3954 in the fifth exon of the *IL-1B* gene. In the new classification, localized aggressive periodontitis may refer to the Stage III or IV and Grade C. It is thought that different outcomes can be obtained depending on the size (31 healthy subjects and 31 localized aggressive periodontitis patients) and the different status of the patients based on the new classification of periodontal diseases.

The *VDR*, a ligand-controlled transcription factor, intervenes the actions of the vitamin D hormone 1,25-dihydroxyvitamin D3 to alter bone mineral homeostasis. *VDR Taq*I gene polymorphism is positioned at exon 9. The current study demonstrated that increased TT genotype and T allele of the *VDR Taq*I polymorphism in healthy subjects were associated with the decreased risk of periodontitis. Similar to the present study, De Brito et al. [19] reported that the patients with any form of C (formerly t) allele (Tt or tt genotypes) were 2.4 times more susceptible to chronic periodontitis than the patients who lacked C allele. However, Gunes et al. [44] contradicted this finding and suggested that *Taq*I polymorphisms of the *VDR* gene were not associated with severe generalized chronic periodontitis in the Turkish population studied. In two meta-analyses published in 2011 [21] and 2012 [22], no association was observed in the Caucasian population, while chronic, but not aggressive, periodontitis cases, in patients among the Asian population were reported to have a weak, significantly higher frequency of the TT genotype and T allele of *Taq*I. The findings of a recently published meta-analysis, in contrast with the above meta-analyses, reveal a link between periodontitis susceptibility and the *VDR Taq*I polymorphism in Caucasian patients under the dominant model without any apparent correlation in Asian participants [45]. The frequency *Taq*I alleles may vary among different populations. It has been shown that the T allele frequency of the *VDR TaqI* polymorphism is lowest among Asians, about of the 8% population, and highest in Caucasians, at 43% [46]. The genetic susceptibility of a subject to periodontitis due to polymorphism might change on account of the ethnicity of the population.

Genetic factors are of great importance in identifying the host's immune response to infection and could account for significant variation in the severity, distribution, and extension of the disease [47-49]. The grade of periodontitis is predicted by direct or indirect evidence of the progression rate of in three levels: Grade A (slow progression), Grade B (moderate progression) and Grade C (rapid progression) [2]. With the given facts, the present study investigated whether SNPs rs1800587, rs1143634 and rs731236 might be related with the progression of periodontal disease in a group of the Turkish population. In our study, healthy individuals and Grade B and Grade C Stage III periodontitis patients were evaluated and compared. T allele of the rs1143634 was linked to patients having Grade B Stage III periodontitis. Our finding was in accordance with da Silva

et al. [14], who revealed that T allele of the rs1143634 was related with an increased risk of periodontal disease in Asians, Caucasians and in a mixed population. The outcome of the present study demonstrated that rs731236 (*Taq*I) polymorphism is significantly associated with Grade B Stage III periodontitis, but not Grade C, in comparison with healthy individuals. In the assessment of associations between the grading of periodontitis and gene polymorphism, *Taq*I polymorphism CT genotype is higher in Grade B periodontitis patients. In a research study conducted by Chantarangsu et al. [50], with a homogeneous Thai population, the *VDR* gene polymorphism was examined according to the severity of chronic periodontitis (no/mild, moderate and severe). The study revealed that there is no relationship between *Taq*I and periodontitis. To the authors' knowledge, this is the first study evaluating susceptibility to periodontitis according to Grade and Stage approach in the classification of periodontitis. A limitation of our study may be the absence of other stages or grades of periodontitis.

CONCLUSION

In conclusion, CC genotype and C allele of *Taq*I polymorphism were associated with enhanced susceptibility to Stage III periodontitis in a Turkish study group. Furthermore, *VDR Taq*I polymorphism may be useful to discriminate between Grade B and Grade C in Stage III periodontitis. Identifying genetic risk factors according to the stage and grade of periodontitis will improve our knowledge about the pathophysiology and can potentially be used in defining individuals at risk of developing disease and predicting its progression rate.

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REFERENCES

1. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018; 89 Suppl 1:S173-S182. doi:10.1002/JPER.17-0721
2. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. Jun 2018;89 Suppl 1:S159-S172. doi:10.1002/JPER.18-0006
3. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000*. Jun 1994;5:78-111. doi:10.1111/j.1600-0757.1994.tb00020.x
4. Greenstein G, Hart TC. Clinical utility of a genetic susceptibility test for severe chronic periodontitis: a critical evaluation. *J Am Dent Assoc*. 2002; 133(4): 452-9; quiz 492-3. doi:10.14219/jada.archive.2002.0203
5. Faist E, Mewes A, Strasser T, et al. Alteration of monocyte function following major injury. *Arch Surg*. Mar 1988; 123(3): 287-92. doi:10.1001/archsurg.1988.01400270021002
6. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol*. 1997; 24(1): 72-7.
7. Karimbux NY, Saraiya VM, Elangovan S, et al. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. *J Periodontol*. Nov 2012; 83(11): 1407-19. doi:10.1902/jop.2012.110655
8. Trevilatto PC, de Souza Pardo AP, Scarel-Caminaga RM, et al. Association of IL1 gene polymorphisms with chronic periodontitis in Brazilians. *Arch Oral Biol*. 2011; 56(1): 54-62. doi:10.1016/j.archoralbio.2010.09.004
9. Schulz S, Stein JM, Altermann W, et al. Single nucleotide polymorphisms in interleukin-1gene cluster and subgingival colonization with Aggregatibacter actinomycetemcomitans in patients with aggressive periodontitis. *Hum Immunol*. 2011; 72 (10): 940-6. doi:10.1016/j.humimm.2011.05.009
10. Armingohar Z, Jorgensen JJ, Kristoffersen AK, Schenck K, Dembic Z. Polymorphisms in the interleukin-1 gene locus and chronic periodontitis in patients with atherosclerotic and aortic aneurysmal vascular diseases. *Scand J Immunol*. 2014; 79(5): 338-45. doi:10.1111/sji.12166

11. Tanaka K, Miyake Y, Hanioka T, Arakawa M. Relationship between IL1 gene polymorphisms and periodontal disease in Japanese women. *DNA Cell Biol*. 2014; 33(4): 227-33. doi:10.1089/dna.2013.2202
12. Zuccarello D, Bazzato MF, Ferlin A, et al. Role of familiarity versus interleukin-1 genes cluster polymorphisms in chronic periodontitis. *Gene*. 2014; 535(2): 286-9. doi:10.1016/j.gene.2013.11.016
13. da Silva FR, Guimarães-Vasconcelos AC, de-Carvalho-França LF, et al. Relationship between -889 C/T polymorphism in interleukin-1A gene and risk of chronic periodontitis: Evidence from a meta-analysis with new published findings. *Med Oral Patol Oral Cir Bucal*. 2017; 22(1): e7-e14.
14. da Silva FRP, Vasconcelos A, de Carvalho França LF, Di Lenardo D, Nascimento HMS, Vasconcelos DFP. Association between the rs1143634 polymorphism in interleukin-1B and chronic periodontitis: Results from a meta-analysis composed by 54 case/control studies. *Gene*. 2018;668:97-106.
15. van de Peppel J, van Leeuwen JP. Vitamin D and gene networks in human osteoblasts. *Front Physiol*. 2014 ; 5(137):1-46.
16. Hayes CE, Nashold FE, Spach KM, Pedersen LB. The immunological functions of the vitamin D endocrine system. Research Support, Non-U.S. Gov't Review. *Cellular and molecular biology*. 2003; 49 (2): 277-300.
17. Thakkinstian A, D'Este C, Eisman J, Nguyen T, Attia J. Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *J Bone Miner Res*. 2004; 19(3): 419-28. doi:10.1359/JBMR.0301265
18. Ji GR, Yao M, Sun CY, Li ZH, Han Z. BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and risk of fracture in Caucasians: a meta-analysis. *Bone*. 2010; 47(3): 681-6. doi:10.1016/j.bone.2010.06.024
19. de Brito Junior RB, Scarel-Caminaga RM, Trevilatto PC, de Souza AP, Barros SP. Polymorphisms in the vitamin D receptor gene are associated with periodontal disease. *J Periodontol*. 2004; 75(8): 1090-5. doi:10.1902/jop.2004.75.8.1090
20. Wang C, Zhao H, Xiao L, et al. Association between vitamin D receptor gene polymorphisms and severe chronic periodontitis in a Chinese population. *J Periodontol*. 2009; 80(4): 603-8. doi:10.1902/jop.2009.080465
21. Deng H, Liu F, Pan Y, Jin X, Wang H, Cao J. BsmI, TaqI, ApaI, and FokI polymorphisms in the vitamin D receptor gene and periodontitis: a meta-analysis of 15 studies including 1338 cases and 1302 controls. Comparative Study Meta-Analysis Review. *J Clin Periodontol*. 2011; 38(3): 199-207. doi:10.1111/j.1600-051X.2010.01685.x
22. Chen LL, Li H, Zhang PP, Wang SM. Association between vitamin D receptor polymorphisms and periodontitis: a meta-analysis. Comparative Study Meta-Analysis Systematic Review. *J Periodontol*. 2012; 83(9): 1095-103. doi:10.1902/jop.2011.110518
23. Mashhadiabbas F, Neamatzadeh H, Nasiri R, et al. Association of vitamin D receptor BsmI, TaqI, FokI, and ApaI polymorphisms with susceptibility of chronic periodontitis: A systematic review and meta-analysis based on 38 case -control studies. *Dent Res J (Isfahan)*. 2018; 15(3): 155-165.
24. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Periodontol*. 2018; 89 Suppl 1:S1-S8. doi:10.1002/JPER.18-0157
25. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. *Acta Odontol Scand*. 1964; 22: 121-35.
26. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. *Acta Odontol Scand*. 1963; 21: 533-51.
27. Tai H, Endo M, Shimada Y, et al. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J Clin Periodontol*. 2002; 29(10): 882-8. doi:10.1034/j.1600-051x.2002.291002.x
28. Li QY, Zhao HS, Meng HX, et al. Association analysis between interleukin-1 family polymorphisms and generalized aggressive periodontitis in a Chinese population. *J Periodontol*. 2004; 75(12): 1627-35. doi:10.1902/jop.2004.75.12.1627
29. Lopez NJ, Jara L, Valenzuela CY. Association of interleukin-1 polymorphisms with periodontal disease. *J Periodontol*. 2005; 76(2): 234-43. doi:10.1902/jop.2005.76.2.234
30. Droździk A, Kurzawski M, Safronow K, Banach J. Polymorphism in interleukin-1beta gene and the risk of periodontitis in a Polish population. *Adv Med Sci*. 2006; 51 Suppl 1:13-7.
31. Sakellari D, Katsares V, Georgiadou M, Kouvatsi A, Arsenakis M, Konstantinidis A. No correlation of five gene polymorphisms with periodontal conditions in a Greek population. *J Clin Periodontol*. 2006; 33(11): 765-70. doi:10.1111/j.1600-051X.2006.00983.x

32. Maria de Freitas N, Imbronito AV, Neves AC, Nunes FD, Pustiglioni FE, Lotufo RF. Analysis of IL-1A(-889) and TNFA(-308) gene polymorphism in Brazilian patients with generalized aggressive periodontitis. *Eur Cytokine Netw*. 2007; 18(3): 142-7. doi:10.1684/ecn.2007.0100
33. Kiani Z, Tavakkol-Afshari J, Hojjat H, et al. Polymorphism of IL-1 alpha(-889) Gene and Its Association with Aggressive Periodontitis. *Iran J Allergy Asthma Immunol*. 2009; 8(2):95-8.
34. Moreira PR, de Sá AR, Xavier GM, et al. A functional interleukin-1 beta gene polymorphism is associated with chronic periodontitis in a sample of Brazilian individuals. *J Periodontal Res*. 2005; 40(4) :306-11. doi:10.1111/j.1600-0765.2005.00801.x
35. Agrawal AA, Kapley A, Yeltiwar RK, Purohit HJ. Assessment of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for chronic periodontitis in Maharashtrian ethnicity. *J Periodontol*. 2006; 77(9): 1515-21. doi:10.1902/jop.2006.050427
36. Moreira PR, Costa JE, Gomez RS, Gollob KJ, Dutra WO. The IL1A (-889) gene polymorphism is associated with chronic periodontal disease in a sample of Brazilian individuals. *J Periodontal Res*. 2007; 42(1): 23-30. doi:10.1111/j.1600-0765.2006.00910.x
37. Guzeldemir E, Gunhan M, Ozcelik O, Tastan H. Interleukin-1 and tumor necrosis factor-α gene polymorphisms in Turkish patients with localized aggressive periodontitis. *J Oral Sci*. 2008; 50(2): 151-159. doi:10.2334/josnusd.50.151
38. Shete AR, Joseph R, Vijayan NN, Srinivas L, Banerjee M. Association of single nucleotide gene polymorphism at interleukin-1beta +3954, -511, and -31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. *J Periodontol*. 2010; 81(1): 62-9. doi:10.1902/jop.2009.090256
39. Parkhill JM, Hennig BJ, Chapple IL, Heasman PA, Taylor JJ. Association of interleukin-1 gene polymorphisms with early-onset periodontitis. *J Clin Periodontol*. 2000; 27(9): 682-9. doi:10.1034/j.1600-051x.2000.027009682.x
40. Majumder P, Panda SK, Ghosh S, Dey SK. Interleukin gene polymorphisms in chronic periodontitis: A case-control study in the Indian population. *Arch Oral Biol*. 2019; 101: 156-164. doi:10.1016/j.archoralbio.2019.03.015
41. Wagner J, Kaminski WE, Aslanidis C, et al. Prevalence of OPG and IL-1 gene polymorphisms in chronic periodontitis. *J Clin Periodontol*. 2007; 34(10): 823-7. doi:10.1111/j.1600-051X.2007.01132.x
42. Feng X, Liu J. Association between IL-1A (-889C/T) polymorphism and susceptibility of chronic periodontitis: A meta-analysis. *Gene*. 2020; 729: 1-7. doi:10.1016/j.gene.2019.144227
43. Yücel O, Berker E, Mescil L, Eratalay K, Tepe E, Tezcan I. Association of interleukin-1 beta (+3954) gene polymorphism and gingival crevicular fluid levels in patients with aggressive and chronic periodontitis. *Genet Couns (Geneva, Switzerland)*. 2013; 24: 21-35.
44. Gunes S, Sumer AP, Keles GC, et al. Analysis of vitamin D receptor gene polymorphisms in patients with chronic periodontitis. *Indian J Med Res*. 2008; 127(1): 58-64.
45. Wan QS, Li L, Yang SK, Liu ZL, Song N. Role of Vitamin D Receptor Gene Polymorphisms on the Susceptibility to Periodontitis: A Meta-Analysis of a Controversial Issue. *Genet Test Mol Biomarkers*. 2019; 23(9): 618-633. doi:10.1089/gtmb.2019.0021
46. Uitterlinden AG, Fang Y, van Meurs JBJ, Pols HAP, van Leeuwen JPTM. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004; 338(2): 143-156. doi:https://doi.org/10.1016/j.gene.2004.05.014
47. Nunn ME. Understanding the etiology of periodontitis: an overview of periodontal risk factors. *Periodontol 2000*. 2003; 32: 11-23. doi:10.1046/j.0906-6713.2002.03202.x
48. Zhang J, Sun X, Xiao L, Xie C, Xuan D, Luo G. Gene polymorphisms and periodontitis. *Periodontol 2000*. 2011; 56(1): 102-124. doi:10.1111/j.1600-0757.2010.00371.x
49. Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. *Periodontol 2000*. 2012; 58(1): 37-68. doi:10.1111/j.1600-0757.2011.00415.x
50. Chantarangsu S, Sura T, Mongkornkarn S, Donsakul K, Torrungruang K. Vitamin D Receptor Gene Polymorphism and Smoking in the Risk of Chronic Periodontitis. *J Periodontol*. 2016; 87(11): 1343-1351. doi:https://doi.org/10.1902/jop.2016.160222