Distribution of Vitamin D Receptor–1056 T/C Polymorphism in Healthy People and Patients with Periodontitis

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Abstract

Periodontitis is a complex chronic infection of the periodontal tissues. One of the risk factors of periodontitis is genetic: vitamin D receptor (VDR)-1056 T/C gene polymorphism, which is involved in bone metabolism and affects immune function in a way that leads to bone resorption in periodontitis.

In this cross-sectional study, we investigated the difference in the distribution pattern of the VDR-1056 T/C gene polymorphism between patients with periodontitis and a healthy control group. The VDR-1056 T/C gene polymorphism was analyzed by the polymerase chain reaction–restriction (PCR) fragment-length polymorphism method with Taq I restriction enzyme digestion.

We found that among all participants with the TC genotype, the VDR-1056 T/C gene polymorphism was present in 44.55% of participants with periodontitis and in 55.6% of the control group. This finding indicated that among patients with periodontitis, the VDR-1056 T/C gene polymorphism was present in 44.5% of those with the TC genotype, in 50.5% of those with the TT genotype, and in 0% of those with the CC genotype. But there was no significant difference in the distributions of VDR-1056 T/C gene polymorphism between patients with periodontitis and the control group (P=1.0).

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Introduction

Periodontitis is a complex chronic infection of the periodontal tissues.[1] Periodontitis causes progressive destruction of the periodontal ligament and alveolar bone and is characterized by increased pocket depth, recession, or both.[2] If periodontitis is left untreated, it can result in loss of periodontal ligament support for attachment to the alveolar bone, which leads to tooth loss.[1] Periodontitis is one of the dental problems found in Indonesia.[3] According to Basic Health Survey (RISKESDAS) in 2013, the prevalence of dental and oral diseases in Indonesian society was 25.9%.[4]

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Periodontitis is a multifactorial disease in that it results from the interaction of bacteria, the host, and environmental factors. Risk factors for periodontitis include smoking, age, genetic profile, stress, and alcohol consumption.[2,5,6] Genes associated with periodontitis include *CD14, IL10, IL1B, MMP, TLR4,* and *VDR.*[2]

Vitamin D has a very important role in regulating calcium and phosphorus metabolism. Vitamin D affects the immune system by inhibiting lymphocyte proliferation, stimulating monocyte differentiation, and promoting secretion of cytokines such as interleukin-2, interferon-C, and interleukin-12. Vitamin D can perform this function by binding to nuclear vitamin D (1,25-dihydroxyvitamin D3) receptors (VDRs); the formed complex subsequently enters cell nuclei and acts as a transcription factor that mediates the active effects of vitamin D.[7] Vitamin D affects development periodontal

disease through immunomodulatory effects and effects on bone mineral density.[8]

Research on *VDR*-1056 T/C gene polymorphisms in periodontitis has been carried out in various countries, but this study has never before been conducted in Indonesia. We investigated the expression of *VDR*-1056 T/C gene polymorphism in healthy people and patients with periodontitis.

Materials and methods

The samples for this study were collected from 50 patients with periodontitis and 50 healthy controls. The DNA samples were extracted from the blood serum and were stored at −20°C in the Oral Biology Laboratory, Faculty of Dentistry, Universitas Indonesia. This study was performed with the written approval (No. 010590717) of the Ethics Committee of the Faculty of Dentistry, University of Indonesia. Ethical clearance number 43/Ethical Approval/FKGUI/VII/2017.

Genotyping of the *VDR*-1056 T/C polymorphism was determined by polymerase chain reaction–restriction (PCR) fragment-length polymorphism assay.[9-11] The first step was DNA amplification with PCR at 95°C for 15 min, 33 cycles of denaturation at 94°C for 30 s, annealing at 66°C for 10 s, extension at 74°C for 30 s, and final extension at 74°C for 5 min. For each reaction, we used 10 µL of MyTaq HS Red Mix; 1 µL of forward primers (5´-CAG AGC ATG GAC AGG GAG CAA-3´) and 1 µL of reverse primers (5´-GCA ACT CCT CAT GGC TGA GGT CTC-3´)[12]; 0.1 µL of DNA template; and 7.9 µL of double-distilled water. The 740 bp PCR product was digested by means of TaqI restriction enzyme, and the result was two fragments (495 bp and 245 bp) for the homozygous normal TT genotype, four fragments (495 bp, 290 bp, 245 bp, and 205 bp) for the heterozygous mutant TC genotype, and three fragments (290 bp, 245 bp, and 205 bp) for the homozygous mutant CC genotype feature. The digested products were electrophoretically separated on 2% agarose gels containing GelRed and visualized with gel doc (Bio-Rad Laboratories, Hercules, CA, USA;).

Results

The *VDR*-1056 T/C polymorphism was present in three genotypes: TT (wild-type, homozygote), TC (variant heterozygote), and CC (mutant homozygote). The Hardy Weinberg test was used to estimate the distribution of this polymorphism in the population. The result of this study was a *p* value of 0.222, which means the population of our sample was consistent with Hardy Weinberg equilibrium.

The CC genotype (mutant homozygote) were not found in the samples from patients with periodontitis or the healthy control group. However, the TC genotype (variant heterozygote) was present 9 samples, 5 from the control group (55.6%) and 4 from patients with periodontitis (44.4%).

The distribution of the *VDR*-1056 T/C polymorphism among the patients with periodontitis and the control group was calculated with Fisher's exact test in SPSS v. 22 (IBM Corporation, Armonk, NY, USA). According to the results of this test, the significance level for genotype was 1.0, and that for allele was 1.0. Both values were significant at *P*>.05, which means there was no significant difference in *VDR*-1056 T/C polymorphism distribution between the periodontitis and control groups.

-Polymerase chain reaction (PCR) product visualization of *VDR*-1056 T/C gene (A). Restriction fragment-length polymorphism (RFLP) visualization product of *VDR* -1056 T/C gene (B).

Variables		Presence of Periodontitis *(n)*		*p*
		Yes	No	
Genotype				1
	TT	46 (92.0%)	45 (90.0%)	
	CC	0 (0.0%)	0 (0.0%)	
	TT	4 (8.0%)	5 (10.0%)	
	Total	50 (100.0%)	50 (100.0%)	
Allele				1
	T	96 (96.0%)	95 (95.0%)	
	C	4 (4.0%)	5 (5.0%)	
	Total	100 (100.0%)	100 (100.0%)	

Table 1. Genotype and allele distributions of the *VDR*-1056 T/C polymorphism among patients with and without periodontitis.

Author	Country	Sample		Disease
		Periodontitis	Control	
This study	Indonesia	TT (50.3%) TC (44.4%) CC (0%) Allele T (50.3%) Allele C (44.4%)	TT (49.5%) TC (55.6%) CC (0%) Allele T (49.7%) Allele C (55.6%)	Periodontitis
Sun et al., 2002[14]	China	TT (75.7%) TC (24.3%) CC (0%) Allele T (87.8%) Allele C (12.2%)	TT (94.9%) TC (5.1%) CC (0%) Allele T (97.4%) Allele C (2.6%)	Early-onset periodontitis
Tachi et al., 2003[13]	Japan	TT (89.2%) TC (10.8%) CC (0%) Allele T (94.6%) Allele C (5.4%)	TT (76.6%) TC (23.4%) CC (0%) Allele T (88.3%) Allele C (11.7%)	Chronic periodontitis
de Brito Júnior et al., 2004[15]	Brazil	TT (33.3%) TC (59.4%) CC (7.3%) Allele T (63%) Allele C (37%)	TT (54.5%) TC (31.8%) CC (13.7%) Allele T (70.4%) Allele C (29.6%)	Periodontal Disease
Li et al., 2008[17]	China	TT (88.2%) TC (11.76%) CC (0%) Allele T (94.1%) Alel C (5.9%)	TT (86.8%) TC (13.2%) CC (0%) Allele T (93.4%) Alel C (6.6%)	Agressive Periodontitis
Wang et al., 2009[16]	China	TT (92.5%) TC (6.5%) CC (0.9%) Allele T (95.8%) Allele C (4.2%)	TT (81.8%) TC (18.2%) CC (0%) Allele T (90.9%) Allele C (9.1%)	Chronic periodontitis
Martelli et al., 2011[12]	Italy	TT (35.6%) TC (52.2%) CC (12.2%) Allele T (61.7%) Allele C (38.3%)	TT (20.0%) TC (41.6%) CC (30.8%) Allele T (46.1%) Allele C (53.9%)	Chronic periodontitis
Chantarangsu et al., 2016[18]	Thailand	TT (83.6%) TC (15.3%) CC (1.1%) Allele T (91.2%) Allele C (8.8%)	TT (84.9%) TC (14.3%) CC (0.8%) Allele T (92.0%) Allele C (8.0%)	Chronic periodontitis
Ho et al., 2017[19]	Taiwan	TT (88.0%) TC (12.0%) CC (0%) Allele T (94.0%) Allele C (5.9%)	TT (93.3%) TC (5.5%) CC (1.2%) Allele T (96.0%) Allele C (3.9%)	Aggressive periodontitis

Table 2. Genotype and allele distributions of the *VDR*-1056 T/C polymorphism in various populations.

Discussion

Periodontitis is a complex, multifactorial disease. The amounts of dental plaque and bacteria that cause severe periodontitis cannot be determined because everyone has a different response to the disease. The immune response to periodontitis, the disease progression, and the process of tissue deconstruction are reasons why the study of the role of genes and their polymorphisms in periodontitis has developed rapidly.[12]

1,23-Dihydroxyvitamin D is the active form of vitamin D, which has a role not only in bone metabolism but also in facilitating both phagocytosis by monocytes and monocyte differentiation, which contribute to the immune response.[13] Vitamin D can strengthen the immune system and can perform this function by binding to nuclear vitamin D receptors (1,25-dihydroxyvitamin D3 [VDR]).[7] Vitamin D deficiency results in reduction of bone mineral density, including that of the maxilla and mandible, through an increase in alveolar bone porosity and rapid bone resorption, all of which is part of the pathogenesis of periodontitis.[12]

Research on *VDR*-1056 T/C gene polymorphisms in periodontitis has been carried out in various countries such as Japan, Brazil, China, Italy, Taiwan, and Thailand but not before in Indonesia. Therefore, our aim was to determine the presence of *VDR*-1056 T/C gene polymorphism and periodontitis in the Indonesian population.

In 2002, Sun et al. found an association between the *VDR*-1056 T/C gene polymorphism and early onset of periodontitis in the Chinese population (*P*=.017).[14] in 2003, Tachi et al. stated that the *VDR*-1056 T/C gene polymorphism and chronic periodontitis were associated in the Japanese population (*P*=.034).[13] In research in the Brazilian population, de Brito Júnior et al. demonstrated the association between the *VDR*-1056 T/C gene polymorphism and periodontal disease (*P*=.016).[15] In 2009, Wang et al. showed that the VDR-1056 T/C gene polymorphism was associated with chronic periodontitis in the Chinese

population (*P*=.019).[16] In 2011, Martelli et al. demonstrated the association between *VDR*-1056 T/C gene polymorphism and chronic periodontitis in the Italian population.[12]

In contrast, research conducted by Li et al. in 2008 did not show an association between the *VDR*-1056 T/C gene polymorphism and aggressive periodontitis in the Chinese population (*P*=.82).[17] Research conducted by Chantarangsu et al. in 2016 also did not reveal any relation between the *VDR*-1056 T/C gene polymorphism and chronic periodontitis in the Thai population (*P*=.09).[18] In research on the *VDR*-1056 T/C gene polymorphism, Ho et al. (2017) showed no correlation between it and aggressive periodontitis in the Taiwanese population (*P*=.11).[19]

In this study, we found that TT genotypes were present in 91 samples: 46 from patients with periodontitis (50.5%) and 45 from healthy controls (49.5%). TC genotypes were present in 9 samples: 4 from patients with periodontitis (44.4%) and 5 from controls (55.6%). In this study, no CC genotypes were found in either periodontitis or control samples. The TT genotype was thus more common in periodontitis samples than in control samples; this finding is consistent with findings in Japan, China, and Italy but different from research in Brazil, Thailand, and Taiwan. In addition, C alleles were more common in control samples than in periodontitis samples. This is consistent with results of research conducted in Chinese, Japanese, and Italian populations but differs from results obtained in Brazil, Thailand, and Taiwan. These different research results may be attributable to race and ethnicity of the different populations.

The distribution of the *VDR*-1056 T/C gene polymorphism among patients with periodontitis and healthy individuals in this study, according to the results of Fisher's exact test, yielded *P*=1.0 in the genotype of the study sample. We can conclude that there was no significant difference in the distribution of the *VDR*-1056 T/C gene polymorphism between patients with periodontitis and healthy individuals. This is possible because of factors that influence periodontitis, such as environmental factors, race and ethnicity, and the habits of each individual (Table 2).

The main limitation of the study was the small sample size. Further studies are suggested for providing more genetic information and revealing risk factors that could have affected the results of this study.

Conclusions

In conclusion, *VDR*-1056 T/C gene polymorphisms were present to equal degrees in patients with periodontitis and in healthy controls (*P*=1.0).

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Declaration of Interest

The authors report no conflict of interest.

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