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Investigating the Association between rs4986790 Polymorphism of TLR4 Gene and Chronic Periodontitis in an Iranian Population

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Objectives Periodontitis is a multifactorial disease in terms of etiology, including microbial, environmental, systemic, and genetic factors, and the host's immune response causes periodontal destruction. The present study was done to investigate the association between rs4986790 polymorphism of the TLR4 gene with chronic periodontitis in an Iranian population.
Method The present case-control study was conducted on 120 people, including two groups of control (n=66) and patient (n=54) in 2022. A periodontist evaluated the periodontal condition of the people, 5 milliliters of blood sample was taken from participants, gene sequence was determined using the PCR-RFLP method, and statistical analysis was performed using the chi-square test to determine the association between the disease and the mentioned polymorphism.
Results 64 participants (53.33%) were male, and the rest were female. The frequency of the A allele was 89% in the patient group and 90% in the control group, and the frequency of the G allele was 11% in the patient group and 10% in the control group. Also, the frequency of AA and AG genotypes was 78% and 22% in patients, and 80% and 20% in the control group, respectively (P=0.65). There was no statistically significant association between the genders, the presence of a specific allele or genotype with the occurrence of the disease. The odds ratio (OR) of the developing periodontitis in the heterozygous group compared to the homozygous group was calculated to be 0.78% (P=0.65).
Conclusion There was no association between TLR-4 gene polymorphism and chronic periodontitis in the studied population.
Keywords TLR4 Receptor; Chronic periodontitis; Single nucleotide polymorphism

Introduction

Periodontitis is an inflammatory disease of tooth supporting tissues with microbial origin. Its most common form is chronic type, which is divided into three categories based on severity: mild, moderate, and severe. [1] The prevalence of this disease in American adults over 30 years old is up to 42%, and its severe form is observed in 7.8% of people. [2] The treatment includes mechanical plaque removal, proper hygiene education [3], and conservative surgical interventions. [4] Without any treatment, the disease progresses. In that case, the pocket depth, bleeding on probing (BOP), bone loss, and tooth loosening increase and can lead to tooth loss [5], which in turn causes functional, aesthetic, psychological, social, and economic problems for the patient. [5] Toll-like receptors (TLRs) are important receptors of the immune system that bind to various ligands and lead to the release of inflammatory cytokines from cells. [6] TLR2 and TLR4 are the main receptors for bacterial cell wall components and play a role in detecting many periodontal pathogens. [7, 8] After detecting the Lipopolysaccharides (LPS) of Gram-negative bacteria, TLR4 specifically produces and releases pro-inflammatory cytokines, which destroy the connective tissue and bone; therefore, polymorphism in the genes encoding TLR4 may play a role in increasing the susceptibility to periodontitis and even other diseases. [9-22]

In various studies, researchers have investigated the association between TLR4 gene polymorphism and the incidence of chronic periodontitis and have presented different results regarding the presence or absence of such association. [13-19] In a study by Li et al. (2019) in China, different polymorphisms of TLR4 were associated with moderate to severe chronic P.g – related periodontitis. [13] Zacharias et al. (2019) also found in a study in Brazil that there was no association between TLR4 gene polymorphism and its receptor with periodontitis. Although it was considered as a risk factor for periodontitis in men. [14] Harish Reddy et al. (2011) found no significant association between TLR4 polymorphism and chronic periodontitis in South India. [15] Schröder et al. (2005), in a study in Germany, found that TLR-4 polymorphism is associated with chronic periodontitis but not with the aggressive type.[16]

Considering the results of the studies above regarding the association between TLR4 gene polymorphism (rs4986790) and chronic periodontitis, which are not adequately clear and contradict each other in some cases, and considering the high prevalence of periodontitis and the lack of identification of its genetic risk factors and also lacking availability of sufficient data about the association between the above-mentioned gene polymorphism and periodontitis in Iran, this study aimed to investigate the association between TLR4 gene polymorphism

(rs4986790) and periodontitis in an Iranian population in 2022. The present research result may contribute to preparing documented data based on academic research to ensure better evaluation of periodontitis, determining disease risk, and better screening.

Methods and Materials

In this case-control analytical study, non-random sampling was carried out by continuously referring patients to medical centers and dental clinics affiliated with Shahid Beheshti, Zanjan, and Mashhad Universities of medical sciences. According to the inclusion criteria, 54 people with chronic periodontitis were included in the study group, and 66 healthy people were assigned to the control group.

Clinical phase: Three final-year general dentistry students in Tehran, Mashhad, and Zanjan performed clinical examinations and blood sampling under the supervision of a trained periodontist. To divide people into case (periodontitis) and control (healthy) groups, the first clinical examinations were carried out using the Williams Probe. The CAL and PD were measured at six points around the tooth using the mentioned probe, and the presence or absence of BOP was also measured. People with BOP, CAL>3 mm, and PD ≥ 4 mm were included in the case group, and the rest were included in the control group. Based on the CAL level, the study group subjects were divided into three subgroups: mild, moderate, and severe. Mild, moderate, and severe periodontitis were represented by CAL<2, 2-4, and > 5 mm, respectively. Other factors, such as gingival color and the presence or absence of mass, were also measured and recorded. After selecting the patients and healthy people based on the inclusion and exclusion criteria, obtaining their informed consent, and assuring them that their information would be kept confidential, 5 ml of venous blood samples were taken from each person and stored in EDTA tubes to be sent to the laboratory. To avoid bias and ensure blinding, each patient was given a code written on the samples. Then, all the samples were sent to the laboratory for related examination and gene sequencing.

Laboratory phase: The samples were centrifuged at 1000 g for 10-15 minutes. Then the blood was separated into three layers: serum, buffy coat, and red blood cells. The buffy coat was used for DNA extraction, and two other layers were not needed. The Pars Toos Company Column Kit used the buffy coat (200 µL) for DNA extraction. PCR-ARMZ technique was used to determine the rs4986790 variant in the TLR-4 gene. For this purpose, the desired SNP was searched from the relevant part in the NCBI database, the desired sequence was found, and the necessary primers for the amplification of this region were designed by Oligo7 software. After confirming the specificity through BLAST in the NCBI database, the prepared sequences were ordered to Pishgam Biotechnology Company for synthesis. The sequence of studied primers is shown in Table 1.

Table 1: Nucleotide sequence of used primers

5’-TGAACCCTATGAACTTTATCC-3’	Outer upper
5’-GTTAACTAATTCTAAATGTTGCCATC-3’	Outer lower
5’-GCATACTTAGACTACTA CCTCGAAGA-3’	Inner upper
5’-CA AACAATTAAATAAGTCAATAATAC-3’	Inner lower

PCR: To amplify the gene sequence, PCR was performed as follows: denaturation for 3 minutes at 94°C and 30 cycles at 94°C for 30 seconds, annealing at 57°C for 50 seconds, extension at 72°C for 40 seconds, and final extension at 72°C for 10 minutes.

After amplification of the desired PCR fragments and juxtaposing them with the indicated primers for cutting, electrophoresis was performed. The DNA with allele A was divided into two fragments of 147bp and 385bp, and if there was a fragment with allele G and the function of the primers, the target sequence (385bp) was broken into two 292bp and 147bp fragments (Figure 1).

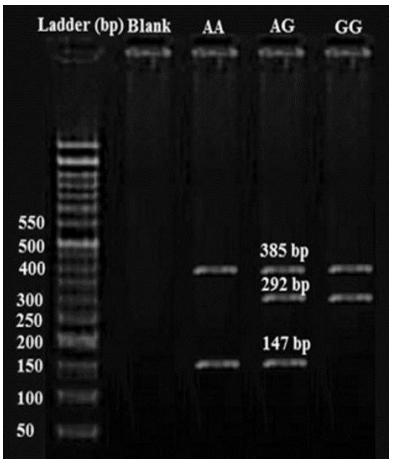

Figure 1: Fragments separated in electrophoresis

Statistical Analysis

The collected data were analyzed using SNPStats online software (www.snpstats.net).

After ensuring Hardy-Weinberg equilibrium in genetic drift, statistical data were evaluated using the chi-square test. The association between genotype frequencies and the type and severity of periodontitis in three inheritance models, including dominant, recessive, and dominant-recessive was investigated using Pearson's chi-square test. The P-value was corrected by multiplying the number of SNPs, and the P-value<0.05 was considered significant. Results were expressed as odds ratio (OR) and 95% confidence interval (95% CI) adjusted P-value. Patients were matched with the control group regarding variables such as BMI.

Ethical Considerations

Sampling and examination were performed for all patients according to the standard protocol and under the supervision of a trained professor. The patients were justified regarding the confidentiality of the research results and the presentation of the results to them upon request, and informed consent was obtained from them. Finally, to appreciate the participants' cooperation, each was given an oral hygiene package containing a toothbrush and toothpaste. In this research, there was no intervention in the treatment process of the patients, and the obtained results were expressed in complete honesty. This study was approved by the Ethics Committee of Dental Sciences Research Institute, Shahid Beheshti University of Medical Sciences, with the ethics code of IR.SBMU.DRC.REC.1400.160.

Results

This case-control study was conducted on 120 samples, including 66 healthy people (55%) and 54 patients with chronic periodontitis (45%). A total of 30 patients (55.5%) and 34 healthy people (51.5%) were male, and the rest were female, and there was no significant difference between the two patient and control groups in terms of gender (p-value>0.05).

Hardy-Weinberg equilibrium was established in the control group based on the genotypic and allelic distribution of TLR4 (rs4986790) polymorphism (P-value=1). The allelic distribution of TLR-4 polymorphism is shown in Table 2.

Table 2- Allelic distribution of TLR-4 polymorphism in patients and healthy people

TLR4 genotype frequencies (n=120)				
Genotype	Control	Chronic Periodontitis	Total	P value
A/A	53 (80%)	42 (78%)	95 (79%)	0.65
A/G	13 (20%)	12 (22%)	25 (21%)	

The genotypic frequency of polymorphism can be observed in Table 3, along with examining different types of inheritance models.

Table 3- Frequency of TLR-4 polymorphisms in patients and healthy people

TLR4 allele frequencies (n=120)			
Allele	Control	Chronic Periodontitis	Total
A	119 (90%)	96 (89%)	215 (90%)
G	13 (10%)	12 (11%)	25 (10%)

The AA and AG frequency was 78% and 22% in patients, and 80% and 20% in the control groups, respectively, which show no significant difference between the two groups in terms of any of the inheritance patterns. The A allele frequency was 89% in the patient group and 90% in the control group, and the frequency of the G allele was 11% in the patient group, and 10% in the control group, and no significant difference was observed between the two groups in this regard.

Detailed examination of Hardy-Weinberg for alleles and genotypes of this polymorphism is shown in Table 4.

The OR of developing periodontitis in the heterozygous group compared to the homozygous group was calculated to be 0.78% (Table 5).

Table 4- Detailed examination of Hardy-Weinberg test for alleles and polymorphism genotypes

TLR4 exact test for hardy-weinberg equilibrium (n=120)						
	AA	AG	GG	A	G	P-value
Total	95 (79%)	25 (21%)	0 (0%)	215 (90%)	25 (10%)	0.36
Chronic Periodontitis	42 (78%)	12 (22%)	0 (0%)	96 (89%)	12 (11%)	
Control	53 (80%)	13 (20%)	0 (0%)	119 (90%)	13 (10%)	

Table 5- Odds ratio of periodontitis in two homozygous and heterozygous models

TLR4 association with response Ca.Co (n=120, adjusted by code)				
Genotype	Chronic Periodontitis	Control	OR (95% CI)	P-value
AA*	42 (77.8 %)	53 (80.3 %)	0.78 (0.26-2.29)	0.65
AG	12 (22.2 %)	13 (19.7 %)		

*: Reference group

Discussion

The present study showed no association between TLR-4 polymorphism (rs4986790) and chronic periodontitis in an Iranian population. The ratio of healthy people and patients with chronic periodontitis was almost the same between

men and women, and there was no association between the gender of people and susceptibility to chronic periodontitis. There was also no significant difference between the case and control groups regarding the genotypic distribution and allele polymorphism in this gene locus considering two alleles, A and G, and two genotypic AA and AG models.

Several studies have reached results that were similar to the present study. Zacharias et al. (2019) [14] investigated the association between the aforementioned polymorphism and the susceptibility to periodontitis in a part of their research on a Brazilian population. A total of 203 patients with periodontitis and 213 healthy individuals were examined as a control group in their study. Subjects underwent clinical periodontal examination, and an experienced periodontist confirmed the diagnosis. In this study, similar to the current study, CAL, BOP, and PD factors were not investigated, but whether people were smokers or non-smokers in both healthy and patient groups was recorded. They also stated that, in general, there is no significant association between the relevant polymorphism and susceptibility to periodontitis. However, they observed that the AG genotype is a risk factor for periodontitis in men, regardless of the smoking variable.

In their study in Turkey, Berdeli et al. (2007) [19] investigated the association between the relevant polymorphism and the susceptibility to chronic periodontitis. The frequency of allele 299Gly was 2.4% and 2.8% in chronic periodontitis and healthy groups, respectively, which showed no significant difference. Also, none of the two groups showed homozygosity for the alleles of this polymorphism. They stated that the percentage of BOP sites with a plaque in patients with the 299Gly allele is significantly higher than those without this allele. Still, no association was observed between this polymorphism and chronic periodontitis, which is similar to the result of the present study. In a German study, Schulz et al. (2021) [23] showed a biological link between severe periodontitis and cardiovascular disease, and both diseases were influenced by genetic background. Therefore, the genetic changes of several innate immune system receptors, including the gene polymorphism studied in the present study, were investigated. A total of 1002 patients with cardiovascular diseases were enrolled in the above study and followed up for 3 years. The effect of these genetic variants on severe periodontitis and the outcome of cardiovascular disease was evaluated. Similar to the result of the present study, it was observed that the mentioned polymorphism plays no regulatory or modulating role for pre-existing cardiovascular diseases or severe periodontitis in these patients. Also, Zheng et al. (2013) [24], in a meta-analysis study, reached a conclusion that was similar to the present study. In the subgroup analysis based on ethnicity and type of periodontitis, the results showed no association. Still, they stated that the risk of periodontitis increased significantly in the recessive inheritance of this polymorphism.

Schröder et al. (2005) [16] investigated the association between polymorphisms of several genes, including the periodontitis gene, in a population of German people. They obtained a result that was contrary to the present study. In that study, it was found that there was an association between the studied polymorphism with the severity of chronic generalized periodontitis, but not the aggressive type. Therefore, the relevant polymorphism was introduced as a risk factor for generalized chronic periodontitis. In their study, smokers were included in the study, unlike the present study. In addition to introducing different inclusion and exclusion criteria, the larger sample size included in the study and the racial difference can also be possible causes of the discrepancy in the results. In this study, CAL was also measured.

On the other hand, the association between the polymorphism of different genes and the susceptibility to periodontitis was investigated in the meta-analysis study by Han et al. (2015). [17] The relevant polymorphism was also investigated and found to have no significant association with chronic and invasive periodontitis. Still, the susceptibility to periodontitis was partially controlled by pattern recognition receptors (PRRs), such as the polymorphism above. At the same time, the presence of the G allele in the Caucasian race could increase the risk of chronic periodontitis by 32%, which is also contrary to the results of our study, and a significant association was observed between the allelic distribution and the incidence of the disease in this race. In that study, unlike the present study which investigated only Iranian people in three cities, patients from several countries such as the Czech Republic, Germany, England, Sweden, etc., as well as from several races such as Caucasian, Asian, African, etc. were enrolled and investigated. The difference in the results can be attributed to this racial difference.

On the other hand, the results of the recent study contradict the results of a study by Sellers et al. (2016) [18] in the United States. Their study investigated the association between the above polymorphism and the susceptibility to chronic periodontitis in patients with rheumatoid arthritis or osteoarthritis. They also measured the P. gingivalis(P.g) count in these people. It was found that this polymorphism significantly interacted with P.g and could even have a protective role against chronic periodontitis and prevent its occurrence. Therefore, this polymorphism was described as a factor in preventing the disease. The sample size of that study was larger than the present study, its inclusion and exclusion criteria were different, and patients, smokers, hypertensive patients, etc. were included, which may justify the discrepancy in the results. The association between different TLR-4 polymorphisms with periodontitis in P.g carriers was investigated in a study on Chinese

people by Li et al. (2019). [13] They did not find this polymorphism in their participants and could not investigate the effect of this particular SNP on susceptibility to the disease. Still, they confirmed the association of other TLR-4 polymorphisms with the disease. Similarly, in a study on the Chinese population, Zhu [25] stated that the frequency of the desired polymorphism is low in the Chinese population. Similarly, in a study on the Indian population, Harish Reddy et al. (2011) [15] reported that the studied polymorphism was not present in their participants, thus they did not evaluate its association with chronic periodontitis. In a study on a Finnish population, Gursoy et al. (2016) [26] investigated the effect of the same polymorphism on alveolar bone loss and its simultaneous interaction with P.g. It was observed that the presence of TLR-4 polymorphism in people and being a salivary P.g carrier simultaneously, is associated with advanced alveolar bone loss. That study did not directly evaluate the effect of the polymorphism mentioned above on chronic periodontitis. Still, since bone loss is directly related to periodontitis disease, the result of the above study can be considered different from the result of the present study. In that study, in addition to the polymorphism mentioned above, the presence of the P.g bacterium was also evaluated, which could be one of the possible reasons for different results.

Chrzęszczyk et al. (2015) [27] investigated the association between the mentioned polymorphism and periodontitis. This meta-analysis reviewed 15 studies with a sample size of 1621 chronic and invasive periodontitis patients and 1755 healthy individuals. Their results showed a possible association between this polymorphism and chronic periodontitis. Unlike the present study, a significant association between this polymorphism and increased susceptibility to chronic periodontitis was shown. Another meta-analysis study by Ozturk et al. [28] also showed that the 299Gly allele increases the odds ratio of developing chronic periodontitis and is a risk factor for the disease. These two studies also included larger sample size and different races, which may justify the discrepancy in the results. Jha et al. (2023) [29] investigated the associations between polymorphism of several genes, including the gene mentioned above, with bacterial infection and gingivitis and oral SCC in India. In that study, 120 cancer patients and 115 healthy cases were investigated, and it was observed that the AG genotype and G allele increased the risk of oral SCC. There are many contradictions between the results obtained in the studies conducted on different populations and even in the same populations. Even in cases where there is an association between these two variables, there are different opinions regarding the type of genotype involved and its role—whether protective or predisposing to the disease.

As we know, periodontitis is a multifactorial disease from an etiological point of view. Despite the efforts made by researchers to make all the samples as uniform as possible and eliminate other intervening and influential factors, some variables are inevitably out of control. Other individual differences such as cellular, molecular, functional, and structural differences within an individual cannot be controlled and are still unknown. Moreover, each of them has affected periodontal health differently and can aggravate the pathogenicity of bacterial agents or fight it. On the other hand, genetic heterogeneity and incomplete penetration of alleles are also more likely to occur. Interactions between different genes and between genes and the environment cannot be controlled and measured and may contribute to explaining different and conflicting results.

The difference in results can also be due to the variable methodology of different studies. Differences in the inclusion and exclusion criteria, the sample size, age groups, and socio-economic variables of the studied populations can all cause some differences. Different sampling methods, the small sample size in some studies, and racial and ethnic differences can also lead to a discrepancy in the results. A larger sample size was included in the studies by Sellers [18], Gursoy [26], and Schröder [16], which reported different results. Different ethnicities, races, and nationalities were included in the studies by Han [17], Chrzęszczyk [27], and Ozturk. [28] However, only the Iranian population was included in the present study. Although people from three different cities were investigated in the present study, which increased the racial diversity compared to Redi's study samples [15] that were collected from only one area.

According to the inclusion and exclusion criteria in the study of Schroeder [16] and Sellers [18], smokers were included in contrast to the present study. Excluding smokers from the present study increased its internal validity but decreased external validity. To access genomic DNA from saliva samples, Gursoy study [26], Lee [13], and Schröder [16] used swabs to take samples from oral mucosa. However, venous blood samples were used to access genomic DNA in the present study, thus there were many problems in terms of ethics, clinical work, and the acceptance of this procedure by patients, which was one of the reasons for the relatively small sample size. Other factors, such as the level of personal hygiene according to the level of culture and education of participants, should not be ignored. Similar results in studies conducted on different populations [14, 19] and the present study, which was conducted for the first time on an Iranian population, may be able to guide further studies while paying special attention to ethnic and racial differences along with genetic factors. Although different results were reported in previous studies conducted in

Germany [16, 17, 23] and some meta-analysis studies. [17, 24, 25]

Different results would have been obtained in the present study if, like the previous studies, the effect of IL-6 polymorphism [30], the effect of IL-2, IL-16, and IL-17 polymorphism [31] and the effect of Vitamin D receptor and vitamin D-binding protein polymorphisms [32] on chronic periodontitis disease were investigated. If so, it would be possible to measure BOP, PPD, and CAL and classify periodontitis in terms of severity (mild, moderate and severe) or even calculate the plaque rate; however, it does not mean that the present study necessarily sought to find a meaningful association, but if investigated, the association between the desired polymorphism with different grades of periodontitis and other mentioned factors could be investigated, which would in turn reduce some of the differences and contradictions in the results. However, many similar articles, such as Zacarias [14], Han [17], and Zheng [24], have not investigated the association between the studied polymorphism with the factors above. These studies showed a significant association between several factors, such as BoP, with polymorphism. Still, they did not show a significant association between polymorphism and the disease incidence.

Overall, according to the foregoing, it is clear that we still do not have a complete understanding of and even complete control over the genetic factors affecting periodontal diseases. However, considering the results of the present study, it may be possible to talk more confidently today about the lack of influence of the above polymorphism on the susceptibility to chronic periodontitis in an Iranian population. However, further studies in other regions of Iran including different Iranian races and ethnicities will surely help us draw more decisive conclusions in this regard. Obviously, the more we know about the role of genetic elements in developing periodontitis, the better and more targeted we will be able to diagnose, treat and control this disease.

To provide sufficient samples to perform the test and to find traces of the genetic effect of the studied polymorphism on the susceptibility to periodontitis, sampling was carried out in three centers in three different cities. The samples were examined and extracted by three different students under the supervision of a periodontist. Thus the probability of error increased. Also, due to the recent COVID-19 pandemic (2020-2021), it was not possible to accurately record information related to periodontal criteria such as PD, BOP, CAL, etc. for all people; therefore, only the sick or healthy status of each person was recorded inevitably. Accordingly, it was impossible to determine the severity of the disease. If we managed to include and record these cases, investigating their association with the desired SNP would obviously make more complete and valuable results. Definitely, the results will be more generalizable if a larger sample size is used and clinical factors are recorded at the national level by dividing the region or province into future studies. It is suggested to carry out the future studies with more accurate methods and other TLR gene loci being investigated at the same time. Unfortunately, these factors were beyond our control due to financial, human, and time limitations.

Conclusion

This study found no association between TLR-4 gene polymorphism and chronic periodontitis. It is suggested to study a larger sample size more accurately and in different regions and racial groups in future.

Conflict of Interest

No Conflict of Interest Declared ■

References

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Northwest Dent. 2000;79(6):31-5.
2. Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA, Genco RJ. Periodontitis in US adults: national health and nutrition examination survey 2009-2014. J Am Dent Assoc. 2018;149(7):576-88.
3. Vahabi S, Nazemi B. A comparison of Chlorhexidine impregnated floss vs. conventional dental floss on gingivitis. J Dent Sch. 2008; 25(4):418-25.
4. Deas DE, Moritz AJ, Sagun RS Jr, Gruwell SF, Powell CA. Scaling and root planing vs. conservative surgery in the treatment of chronic periodontitis. Periodontol 2000. 2016;71(1):128-39.
5. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. Periodontol 2000. 2012;60(1):15-39.
6. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5:1-8.
7. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999;11(4):443-51.
8. Swaminathan V, Prakasam S, Puri V, Srinivasan M. Role of salivary epithelial toll-like receptors 2 and 4 in modulating innate immune responses in chronic periodontitis. J Periodontal Res. 2013;48(6):757-65.
9. Yoshimura A, Kaneko T, Kato Y, Golenbock DT, Hara Y. Lipopolysaccharides from periodontopathic bacteria porphyromonas gingivalis and capnocytophaga ochracea are antagonists for human toll-like receptor 4. Infect Immun. 2002;70(1):218-25.
10. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2(8):675-80.
11. Costa AM, Guimarães MC, de Souza ER, Nóbrega OT, Bezerra AC. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. Gerodontology. 2010;27(1):70-5.

12. Archana PM, Salman AA, Kumar TS, Saraswathi PK, Panishankar KH, Kumarasamy P. Association between interleukin-1 gene polymorphism and severity of chronic periodontitis in a south Indian population group. J Indian Soc Periodontol. 2012;16(2):174-8.
13. Li W, Cao X, He L, Meng H, Yang B, Liao Y. TLR4 polymorphisms may increase susceptibility to periodontitis in pg-positive individuals. PeerJ. 2019;7:e7828.
14 .Zacarias JMV, de Alencar JB, Tsuneto PY, de Souza VH, Silva CO, Visentainer JEL, et al. The influence of TLR4, CD14, OPG, and RANKL polymorphisms in periodontitis: a case-control study. Mediators Inflamm. 2019;2019:4029217.
15. Reddy BH, Jayakumar ND, Akula SR, Sharma R, Kaarthikeyan G, Sankari. Analysis of association between TLR-4 Asp299Gly and Thr399Ile gene polymorphisms and chronic periodontitis in a sample of south Indian population. J Indian Soc Periodontol. 2011;15(4):366-70.
16. Schröder NW, Meister D, Wolff V, Christan C, Kaner D, Haban V, et al. Chronic periodontal disease is associated with single-nucleotide polymorphisms of the human TLR-4 gene. Genes Immun. 2005;6(5):448-51.
17. Han MX, Ding C, Kyung HM. Genetic polymorphisms in pattern recognition receptors and risk of periodontitis: evidence based on 12,793 subjects. Hum Immunol. 2015;76(7):496-504.
18. Sellers RM, Payne JB, Yu F, LeVan TD, Walker C, Mikuls TR. TLR4 Asp299Gly polymorphism may be protective against chronic periodontitis. J Periodontal Res. 2016;51(2):203-11.
19. Berdeli A, Emingil G, Han Saygan B, Gürkan A, Atilla G, Köse T, et al. TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms are not associated with chronic periodontitis in a Turkish population. J Clin Periodontol. 2007;34(7):551-7.
20. Mohammadpour-Gharehbagh A, Jahantigh D, Eskandari M, Eskandari F, Rezaei M, Zeynali-Moghaddam S, et al. The role of TNF-α and TLR4 polymorphisms in the placenta of pregnant women complicated by preeclampsia and in silico analysis. Int J Biol Macromol. 2019;134:1205-15.
21. Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, et al. Associations of a TLR4 single-nucleotide polymorphism with H. pylori associated gastric diseases in Iranian patients. Clin Res Hepatol Gastroenterol. 2014;38(3):366-71.
22. Bahrami H, Daneshmandi S, Heidarnazhad H, Pourfathollah AA. Lack of association between toll like receptor-2 and toll like receptor-4 gene polymorphisms and other feature in Iranian asthmatics patients. Iran J Allergy Asthma Immunol. 2015;14(1):48-54.
23 Schulz S, Zielske M, Schneider S, Hofmann B, Schaller HG, Schlitt A, et al. Polymorphism of CD14 gene is associated with adverse outcome among patients suffering from cardiovascular disease. Mediators Inflamm. 2021;2021:3002439.
24. Zheng J, Gao L, Hou T, Liu Z, Wu C, Wu S, et al. Association between TLR4 polymorphism and periodontitis susceptibility: a meta-analysis. Crit Rev Eukaryot Gene Expr. 2013;23(3):257-64.
25. Zhu G, Li C, Cao Z, Corbet EF, Jin L. Toll-like receptors 2 and 4 gene polymorphisms in a Chinese population with periodontitis. Quintessence Int. 2008;39(3):217-26.
26. Gursoy UK, He Q, Pussinen P, Huumonen S, Könönen E. Alveolar bone loss in relation to toll-like receptor 4 and 9 genotypes and porphyromonas gingivalis carriage. Eur J Clin Microbiol Infect Dis. 2016;35(11):1871-6.
27. Chrzęszczyk D, Konopka T, Ziętek M. Polymorphisms of toll-like Receptor 4 as a risk factor for periodontitis: meta-analysis. Adv Clin Exp Med. 2015;24(6):1059-70.
28. Ozturk A, Vieira AR. TLR4 as a risk factor for periodontal disease: a reappraisal. J Clin Periodontol. 2009;36(4):279-86.
29. Jha A, Nath N, Kumari A, Kumari N, Panda AK, Mishra R. Polymorphisms and haplotypes of TLR-4/9 associated with bacterial infection, gingival inflammation/recession and oral cancer. Pathol Res Pract. 2023;241:154284.
30. Salman BN, Vahabi S, Biglari A, Salavitabar S, Doabsari MH. Correlation of interleukin-6-174 GC and interleukin-6-572 GC gene polymorphisms with periodontal disease in an Iranian population. *Dent Res J* (Isfahan). 2016;13(4):354-61.
31. Vahabi, S, Nazemisalman, B, Hosseinpour, S, Salavitabar, S, Aziz, A. Interleukin-2, -16, and -17 gene polymorphisms in Iranian patients with chronic periodontitis. J Invest Clin Dent. 2018;9(2):e12319.
32. Nazemisalman B, Vahabi S, Sabouri E, Hosseinpour S, Doaju S. Association of vitamin D binding protein and vitamin D receptor gene polymorphisms in Iranian patients with chronic periodontitis. Odontology. 2019;107(1):46-53.

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