

Original Article

Correlation of interleukin-6-174 GC and interleukin-6-572 GC gene polymorphisms with periodontal disease in an Iranian population

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ABSTRACT

Background: Periodontal disease has a multifactorial etiology. A combination of microbial agents and environmental, habitual, systemic, and genetic risk factors is responsible for the development of periodontal disease. Host immune response causes the destruction of tooth-supporting structure and eventual tooth loss. This study aimed to assess the correlation of interleukin 6 (IL-6) -174-GC and IL-6-572-GC gene polymorphisms with periodontal disease in an Iranian population.

Materials and Methods: This case-control analytical study was conducted on 129 subjects presenting to the laboratory of Taleghani Hospital. Subjects underwent clinical and periodontal examinations and divided into five groups of healthy, gingivitis and mild, moderate and severe periodontitis. Blood samples (2 ml) were obtained. Genomic DNA was extracted manually using the salting-out method. IL-6 sequence amplification was performed using polymerase chain reaction with three thermal protocols. Digested products were analyzed by electrophoresis through 2% agarose gel using Gel Red staining. Data were analyzed using Chi-square, Kruskal-Wallis, and Mann-Whitney tests, and $P < 0.05$ was considered significant.

Results: The frequency of GG polymorphism at IL-6-174 and IL-6-572 genomic regions was 51.2% and 71.3%, respectively. The frequency of IL-6-572-GG polymorphism was significantly greater than that of IL-6-572-GC polymorphism ($P < 0.001$). Comparison of the mean and maximum pocket depth and clinical attachment level, as well as bleeding on probing percentage, revealed significant differences between the healthy controls and periodontitis patients ($P < 0.001$). The frequency percentages of GC and GG polymorphisms were almost equal in the healthy, gingivitis, and periodontitis groups. In other words, the frequency of the two polymorphisms was not significantly different between the health and disease states ($P = 0.065$ for IL-6-572 and $P = 0.63$ for IL-6-174).

Conclusion: This study found no association between IL-6-174 and IL-6-572 gene polymorphisms and periodontitis in the studied population.

Key Words: Interleukin-6, periodontal disease, polymorphism

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INTRODUCTION

Periodontal disease is an infection of the tooth supporting structure and is divided into two main

groups of gingivitis and periodontitis.^[1] Chronic periodontitis is the most common form of periodontitis

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that affects up to 30% of adults. In 7–13% of patients, periodontal disease progresses to a severe form.^[2] Periodontal disease is initiated by the accumulation of bacterial plaque in the gingival sulcus triggering immune response.^[3] The first step in prevention and treatment of periodontal disease is mechanical plaque removal and proper oral hygiene instructions. If not treated, progression of the disease leads to increased pocket depth (PD), bleeding, bone loss, tooth mobility, and eventual tooth loss.^[4] Destruction of tooth supporting structure and alveolar bone occurs as the result of host immune response to bacteria and their products.^[3] Although oral microbial flora and environmental factors affect the pathogenesis of periodontal disease,^[5] evidence shows that genetics can also be a risk factor.^[6]

Cytokines namely interleukin (IL) -6, IL-1 and tumor necrosis factor- α play important role in the immunopathology of periodontal disease.^[7] Thus, the genetic regulation of cytokine function may influence the severity of periodontitis. Different types of cells secrete IL-6^[8] and its level of secretion are influenced by the type of cell, type of stimulant, and the underlying genetic mechanism.^[9] IL-6 is an important inflammatory mediator and has both pro- and anti-inflammatory properties.^[10] Single-nucleotide gene polymorphisms of -572 G/C and -174 G/C which are common polymorphisms in the IL-6 gene promoter affect the expression of IL-6 and increase its serum level causing its higher transcription and consequently greater induced response.^[11] Increased IL-6 serum level is correlated with inflammatory diseases like periodontitis.^[12]

Only a few researchers have attempted to assess the relationship of IL-6 and periodontitis worldwide and number of studies conducted in this respect in Iran is scarce.

Costa *et al.* in Brazil concluded that IL-6-174 G/C gene polymorphism may play a role in chronic periodontitis^[13] However, Sanchooli *et al.* in their study in Iran rejected this association.^[14] Tervonen *et al.* concluded that IL-6-174 G/C gene polymorphism had the greatest impact on the development of periodontal disease.^[15] Jingjin *et al.* in their study on the Chinese population concluded that IL-6-572 G/C polymorphism increased the susceptibility of patients to periodontitis.^[16]

Considering the existing controversy regarding the correlation of IL-6 with periodontitis, this study

aimed to assess the correlation of IL-6-572 G/C and -174 G/C gene polymorphisms with periodontal disease in an Iranian population of patients presenting to Taleghani Hospital in 2012.

MATERIALS AND METHODS

This analytical case–control study assessed the relationship of IL-6-572 G/C and -174 G/C gene polymorphisms with periodontal disease in an Iranian population. A total of 129 patients presenting to the laboratory of Taleghani Hospital with a mean age of 34.14 ± 11.81 years were randomly selected including 48 males with a mean age of 35.23 ± 12.46 years and 81 females with a mean age of 33.49 ± 11.48 years.

Considering the multifactorial nature of periodontal disease and the effect of underlying diseases and systemic and environmental conditions on development and progression of periodontitis, the following inclusion and exclusion criteria were set for patients:

All understudy subjects had to be nonsmoker with no systemic disease or predisposing conditions for periodontitis such as diabetes. Patients had to be HIV negative with no history of hepatitis, chemotherapy, radiotherapy, immunosuppression, immune diseases, leukemia, immunosuppressive infections, and malnutrition. Pregnant or nursing patients were also excluded. Having orthodontic appliances, removable denture, acute necrotizing ulcerative gingivitis, mouth opening limitation, gingival surgery, oral hard or soft tissue diseases excluding caries and periodontitis, and taking cyclosporine, Nifedipine, nonsteroidal anti-inflammatory drugs or antibiotics in the past 2 weeks prior to the study were also among the exclusion criteria. Moreover, patients had to have at least 14 teeth in their mouth.

Periodontal health of patients was evaluated by clinical examination and assessment of PD, and clinical attachment level (CAL) in 6 points around each tooth using Williams probe and a dental mirror. Bleeding on probing (BOP), gingival color, and presence of calculus were also assessed by a dentist under the supervision of a specialist for each individual in the laboratory of Taleghani Hospital according to the criteria for periodontal parameters described in periodontology reference textbooks.^[17]

After consultation with a statistician and based on the inclusion and exclusion criteria, patients were divided into five groups:

1. Healthy group: These patients included 15 males and 28 females and did not have any sign of periodontal disease (CAL = 0, PD <3 mm)
2. Gingivitis group: These patients included one male and four females, had CAL = 0 but had periodontal pocket. Gingival color in these patients had turned dark pink or reddish from coral pink in >30% of the areas. The factor of changing color from light pink to dark pink and red in >30% of the regions was used for diagnosing gingivitis or gum tissue inflammation. It is clear that it is possible for teeth to have CAL (nevertheless the color of gum is normal) and suffer from periodontitis. Therefore, the criteria for measurement of PD and CAL are used for making a diagnosis of periodontitis. The change in color in the absence of PD and CAL is used for making a diagnosis of gingivitis
3. Mild periodontitis group: This group included 9 males and 12 females. CAL < 2
4. Moderate periodontitis group: This group included 12 males and 25 females. 2 < CAL < 4
5. Severe periodontitis group: This group included 11 males and 12 females. CAL > 5.

Genomic DNA was extracted from blood lymphocytes from each patient. Blood samples were taken by a laboratory technician using 5 cc vacuum syringes and were transferred to test tubes containing Ethylene Diamine Tetra Acetic acid. Patients were thoroughly informed about the study, written informed consent was obtained and Ethical Committee of Shahid Beheshti School of Dentistry, Tehran, Iran confirmed the study. Patients were ensured about the confidentiality of their information. All patients received free periodontal examination followed by standard oral hygiene instructions. Moreover, each patient received free toothbrush and toothpaste.

Analysis of interleukin-6-572 and interleukin-6-174 gene polymorphisms

Two milliliters of heparinized peripheral blood were obtained from each patient according to Miller *et al.* technique.^[18] The quality and the quantity of DNA were confirmed by agarose gel electrophoresis and spectrophotometry, respectively. IL-6 sequence amplification was done using polymerase chain reaction (PCR) with 3 thermal protocols of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s. This cycle was repeated 35 times. IL-6-174 G/C and -572 G/C polymorphic sites were treated with (5-TGACTTCAGCTTTACTCTTTGT-3 (F)) and (5-CTGATTGGAAACCTTATTAAG-3(R))

and (5-AGATTCCAAGGGTCACTTG-3 (F)) and (5-AGAAGCAGAACCACTCTTC-3 (R)) primers to generate and amplify 198 and 519 base-pair fragments [Figures 1 and 2]. These fragments were then digested overnight at 37°C using SfaNI and BsrBI enzymes (New England Biolabs, Beverly, MA, USA). The digested products were analyzed by electrophoresis through 2% agarose gel and visualized by Gel Red staining.

All phases of DNA extraction and PCR were carried out by a laboratory technician under the supervision of a specialist in the Cellular and Molecular Research Center of Zanjan University of Medical Sciences. After collection, the specimens were frozen at -20°C and transferred to a box containing dry ice 1-month later. Data were analyzed using Chi-square, Kruskal-Wallis and Mann-Whitney tests and $P < 0.05$ was considered significant.

RESULTS

This cross-sectional study was conducted on 129 patients from hospitals in North of Tehran, Iran with a mean age of 34.14 ± 11.81 years including 81 females with a mean age of 33.49 ± 11.48 years and 48 males with a mean age of 35.23 ± 12.46 years. The mean and maximum CAL and PD, as well as the percentage of BOP, were calculated for each patient to diagnose gingivitis or periodontitis. The collected information regarding the clinical periodontal parameters of patients is shown in Table 1.

For each patient, the polymorphism status of the IL-6-174 and IL-6-572 regions was determined as C/C, G/C or G/G. The results showed that only one patient

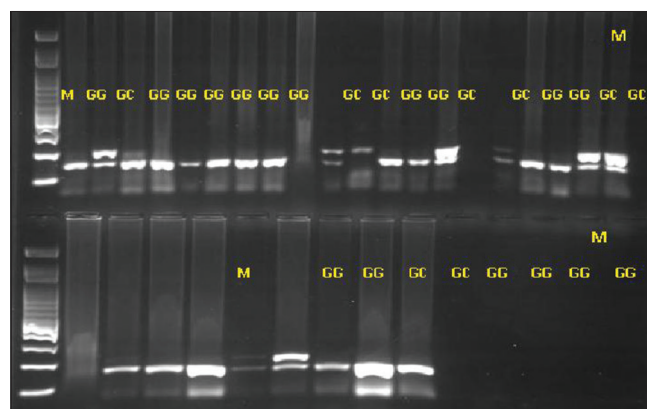


Figure 1: Two percent agarose gel electrophoresis with Gel Red staining for G174C polymorphism. After digestion by SfaNI, for CC, we have No break in the polymerase chain reaction product, but for GG we have two bands (140 and 58 base-pair) and finally for GC we have three bands (198, 140, and 58 base-pair).

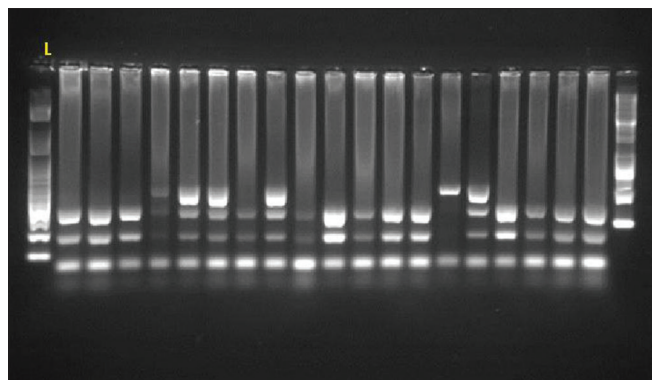


Figure 2: Percent gel electrophoresis Red C572G e.

had IL-6-174 C/C polymorphism, and one other patient had polymorphism C/C polymorphisms at the respective regions, the CC group was eliminated, and all statistical analyses were done on the remaining 129 patients had IL-6-174-GG polymorphism, and 93 patients (71.3%) had IL-6-572-eL-6-572-GG polymorphism was significantly higher than that of IL-6-572-GC polymorphism ($P < 0.001$).

Furthermore, the mean CAL and PD, max CAL, maximum PD and BOP% were compared between males and females and the two polymorphism groups [Table 2a]. First, CAL and PD values were compared between groups. The CAL and PD data had a normal distribution in males and females and also in both polymorphism groups. Thus, the Mann–Whitney test was applied. A significant difference was found in terms of CAL between males and females and males had a significantly higher mean CAL. However, the mean PD was not significantly different between males and females.

Regarding the occurrence of polymorphisms and the relationship between the two types of polymorphisms [Table 2b], it was concluded that regardless of the type of polymorphism at position -174 of IL-6 gene, about 1/3 of patients had GC and 2/3 had GG polymorphism. In other words, if the polymorphism at position-174 of IL-6 gene is of GG type, about 2/3 of patients (72%) have GC polymorphism at position-572 of IL-6 gene. Furthermore, if the polymorphism at position-174 of Iee6 e/3 (72%) of patients have GG polymorphism at position-572 of IL-6 gene.

No significant difference existed between the frequencies of GG and GC polymorphisms at position-174 of IL-6 gene [Table 2c]. No significant difference existed between the frequencies of GG and GC polymorphisms at position -572 of IL-6 gene [Table 2d].

Table 1: The mean and maximum CAL and PD and the percentage of BOP in males and females

Sex	CAL	PD	Maximum CAL	Maximum PD	BOP %
Females					
Mean	1.75	1.84	2.91	3.2	10.47
SD	0.93	0.29	0.74	1.67	
Males					
Mean	2.16	1.95	3.13	3.71	13.02
SD	0.77	0.36	1.14	1.87	
Total					
Mean	1.91	1.88	3.02	3.39	19.27
SD	0.89	0.32	0.91	1.76	

CAL: Clinical attachment level; PD: Pocket depth; BOP: Bleeding on probing; SD: Standard deviation

Table 2a: The mean CAL and PD in males and females

Sex	Number	Mean	SD	P
CAL				
Males	48	2.16	0.1	0.012
Females	81	1.75	0.1	
PD				
Males	48	1.95	0.03	0.059
Females	81	1.84	0.05	

CAL: Clinical attachment level; PD: Pocket depth; SD: Standard deviation

Table 2b: The frequency distribution of GC and GG polymorphisms at position -174 and -572 of IL-6 gene

	-174 position		-572 position		Total
	GC	GG	GC	GG	
GC					
Number	18				63
Line%	28				100
GG					
Number	19	47			66
Line%	28	72			100
Total					
Number	37	92			129

IL-6: Interleukin-6

Table 2c: The mean CAL and PD in subjects with GG and GC polymorphisms at position -174 of IL-6

Periodontal parameters according to genotype, number mean, SD and significance	Genotype	Number	Mean	SD	P
CAL	GG	66	1.95	0.1	0.6
	GC	63	1.86	0.11	
PD	GG	66	1.88	0.03	0.99
	GC	63	1.88	0.045	

CAL: Clinical attachment level; PD: Pocket depth; SD: Standard deviation; IL-6: Interleukin-6

Comparison of the mean CAL and PD among the five groups in terms of disease status revealed significant

differences. Maximum CAL, maximum PD, and BOP% were significantly different among the five groups [Table 3].

Comparison of the intensity of periodontal disease between subjects with the two polymorphisms revealed that in each group, GG and GC polymorphisms

Table 2d: The mean CAL and PD in subjects with IL-6-572 G/G and IL-6-572 G/C polymorphisms

Periodontal parameters according to sex, number mean, SD and significance	Genotype	Number	Mean	SD	P
CAL	GG	92	1.86	0.13	0.36
	GC	37	2.02	0.09	
PD	GG	92	1.87	0.04	0.52
	GC	37	1.91	0.034	

SD: Standard deviation; CAL: Clinical attachment level; PD: Pocket depth; IL-6: Interleukin-6

Table 3: Comparison of the mean (±SD) PD, CAL, maximum CAL, maximum PD and BOP % among the five groups in terms of periodontal disease status

Periodontal parameters according to severity of periodontal diseases	Mean	SD	Median	P
CAL				
Healthy	1.23	0.12	11.5	<0.001
Gingivitis	1.37	0.32	1.41	
Mild periodontitis	1.85	0.13	1.94	
e	2.14	0.07	2.11	
e	2.97	0.12	3.05	
PD				
Healthy	1.67	0.03	1.66	<0.001
Gingivitis	1.82	0.1	1.83	
Mild periodontitis	2.05	0.04	2.05	
Moderate periodontitis	1.88	0.04	1.86	
Severe periodontitis	2.1	0.07	2.14	
Maximum CAL				
Healthy	1.74	0.2	2	<0.001
Gingivitis	2.04	0.2	3	
Mild periodontitis	3.05	0.14	3	
Moderate periodontitis	4.16	0.1	3	
Severe periodontitis	5.74	0.18	3	
Maximum PD				
Healthy	2.44	0.08	2	<0.001
Gingivitis	2.6	0.54	2	
Mild periodontitis	3.29	0.46	3	
Moderate periodontitis	3.14	0.7	4	
Severe periodontitis	3.7	1.34	6	
BOP %				
e	2.1	0.8	0	<0.001
Gingivitis	26.22	15	13.8	
Mild periodontitis	22.13	5.4	8.3	
Moderate periodontitis	14.93	3.4	8.3	
Severe periodontitis	10	3.3	3.3	

CAL: Clinical attachment level; PD: Pocket depth; BOP: Bleeding on probing; SD: Standard deviation

had almost similar frequency percentages. Thus, a significant difference in the incidence of disease between the two groups of polymorphisms seems unlikely. This finding was confirmed by Chi-square test [Table 4].

As seen in Table 4, except for subjects with mild periodontitis who had equal percentage of both types of polymorphisms, in the remaining groups a higher percentage of subjects had GG polymorphism and the difference in this regard was close to significant ($P = 0.065$).

Considering the fact that different degrees of periodontal disease (mild, moderate, and severe) are not distinct, the frequencies of IL-6-174 and IL-6-572 polymorphisms were not significantly different when the healthy group was compared with other groups.

DISCUSSION

This study showed that most subjects had GG polymorphism at position -174 and -572 of IL-6 gene. In other words, the frequency of GG polymorphism at position -174 and -572 of IL-6 gene was higher than that of GC polymorphism. However, our results revealed that no correlation existed between the IL-6-174 and IL-6-572 polymorphisms and various degrees of periodontal disease. In other words, the presence of IL-6 gene polymorphism at the mentioned positions is not a predisposing factor for periodontitis among the Iranian population. This finding is in contrast to the results of Jingjin *et al.*,^[16] Trevilatto *et al.*,^[19] Franch-Chillida *et al.*,^[20] Moreira *et al.*,^[21] and Kalburgi *et al.*,^[22] Shao *et al.*,^[23] Robati *et al.*,^[24] Stefani *et al.*,^[25] and in accordance with the findings of Sanchooli *et al.*^[14]

Our results were different from those of Trevilatto *et al.*^[19] They evaluated the correlation of IL-6-174 polymorphism and susceptibility to chronic periodontitis in a Brazilian population. They analyzed samples for polymorphism using PCR-restriction fragment length polymorphism. In their study, genomic DNA was obtained from the buccal epithelial cells. The difference between our results and those of Trevilatto *et al.*^[19] may be due to the difference in the understudy population and the molecular genetic technique applied.

This study was different from that of Jingjin *et al.*^[16] The age range of subjects in the two groups of chronic periodontitis and controls in their study

Table 4: The frequency percentage of GC and GG genotypes in IL-6-174 and IL-6-572 polymorphisms in different groups in terms of disease status

Genotypes according to severity of periodontal diseases	Severity of disease					Total
	Healthy	Mild	Moderate	Severe	Gingivitis	
IL-6-174 polymorphism						
GC						
Number	23	11	18	8	3	63
Percentage	53.5	52.4	48.6	34.8	60	
GG						
Number	20	10	19	15	2	66
Percentage	46.5	47.6	51.4	65.2	40	
Total	43	21	37	23	5	129
Percentage	33.3	16.3	28.7	17.8	3.9	100
IL-6-572 polymorphism						
GC						
Number	8	11	9	8	1	37
Percentage	18.6	47.6	24.3	34.8	20	
GG						
Number	35	10	28	15	4	92
Percentage	81.4	52.4	75.7	65.2	80	
Total	43	21	37	23	5	129
Percentage	33.3	16.3	28.7	17.8	3.9	100

IL-6-174 polymorphisms: $\chi^2=2.54$, $df=1$, $P=0.63$; IL-6-572 polymorphisms: $\chi^2=8.84$, $df=4$, $P=0.065$. IL-6: Interleukin 6

was 25–65 years; different age range of subjects and ethnicities may explain the difference between the results of these studies.

Our results were also in contrast to those of Franch-Chillida *et al.*^[20] They clinically evaluated 251 systemically healthy Indians and divided them into two groups of healthy controls and periodontitis patients according to the criteria set by the European Workshop on Periodontitis. They evaluated patients' age and CAL and genomic DNA was extracted from oral mucosal cells. The difference between our results and those of Franch-Chillida *et al.*^[20] may be attributed to ethnic differences and use of different diagnostic periodontal parameters.

Our results were not in accordance with those of Moreira *et al.* either.^[21] They evaluated subjects in terms of the severity of periodontal disease and divided them into several groups based on the mean CAL. They reported + G genotype in nonsmoker patients with severe periodontitis; while the frequency of –G genotype was 8.5–9.6% in control subjects and those with moderate periodontitis. Ethnic differences and other environmental factors may be responsible for such variability in results.

Our study yielded results different from those of Kalburgi *et al.*^[22] They used PCR with pfu and screened chronic periodontitis patients with

PD >5 mm and CAL >3 mm in >20 teeth. Ethnic differences, environmental factors, and different sample size may explain the variable results.

Our study was in agreement with that of Sanchooli *et al.*^[14] They reported no association between IL-6-174 gene polymorphism and chronic periodontitis. Furthermore, the frequency of G allele was not significantly different between the two groups, and they concluded that this gene probably plays no role in the development of periodontitis. Similar ethnicity of patients and almost similar sample size may be responsible for this agreement.

Our results were also in accordance with those of a meta-analysis by Shao *et al.*^[23] They evaluated the results of studies on 1093 periodontitis patients and 574 healthy controls and reported that the G allele of IL-6-174 gene polymorphism cannot change the risk of developing chronic periodontitis. IL-6-572 G>C polymorphism was correlated with periodontitis (both chronic and aggressive types).

Our study was different from that of Robati *et al.*^[24] which has been performed in Iran. They evaluated the results of the study on 50 patients including 25 patients with generalized aggressive periodontitis and 25 with healthy periodontium. They determined the level of IL-6 with enzyme-linked immunosorbent assay test. Their results confirmed the association

between generalized aggressive periodontitis and high level of IL-6 as a proinflammatory cytokine; however, they had a limited sample size.

Our study was not in agreement with that of Stefani *et al.*^[25] They used gingival biopsies from 21 patients with chronic periodontitis and 21 controls. Histologic sections stained by hematoxylin-eosin were used for histopathological evaluation. For PCR, they used restriction endonuclease digestion (Hsp II). They deduced the high expression of IL-6 is an important factor related to chronic periodontitis, but were not associated with methylation status or the -174 (G/C) genetic polymorphism. Ethnic differences and different kind of sampling may be responsible for different results.

Considering the lack of scientific evidence in this respect, this study aimed to assess the relationship of IL-6-174 and IL-6-572 gene polymorphisms with periodontal disease in an Iranian population. It should be noted that our study investigated the correlation of different levels of periodontal disease with two IL-6 gene polymorphisms based on gender and age and, number of similar studies is scarce. In order to assess the correlation of IL-6 gene polymorphism and periodontal status, we evaluated the periodontal status of patients using different periodontal parameters including Plaque indexBOP, CAL and PD around each tooth. More favorable results could have been obtained if this study had been done in several provinces of Iran on a larger sample size or if understudy subjects had been examined under standard conditions (optimal for dental examination) and their plaque index had been evaluated as well.

This study had some limitations. Recruiting subjects was difficult considering the need for obtaining blood samples and requiring patient cooperation. Obtaining the required PCR kit was also difficult.

Persians are a mixed race-ethnic group. Thus, other alleles are recommended to be evaluated in future studies since they may play a role in increasing patient susceptibility to periodontal disease. Future studies should be preferably conducted on a mixed sample size from different provinces of Iran.

CONCLUSION

This study found no association between IL-6-174 and IL-6 gene polymorphisms in an Iranian population or between IL-6 gene polymorphisms and periodontal

health or disease states. Future studies with a larger sample size are required to be performed in different geographical locations and on different ethnic populations to assess the correlation of IL-6 gene polymorphism and that of other genes.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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