

## The Relationship between Interleukin-6 -174 G/C Gene Polymorphism and Chronic Periodontitis

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### Abstract

**Background:** Chronic periodontitis is an inflammatory disease that causes rapid destruction of the tissues supporting the teeth. Genetic and environmental factors are involved in its occurrence. It has been suggested that IL-6 promoter gene polymorphism could affect the severity of chronic periodontitis. This study has examined the relationship between IL-6 (-174G/C) gene polymorphism and chronic periodontitis.

**Materials and Methods:** In this case-control study, 100 patients with chronic periodontitis and 100 healthy individuals referring to the clinic of Zahedan Dental School were evaluated. Two ml of peripheral blood was taken from these people. After DNA extraction through salting out method, IL-6 gene polymorphism was determined through T-ARMS-PCR technique using specific primers. The data were analyzed by chi-square test and  $p < 0/05$  was considered significant.

**Results:** The frequency of genotypes CC, GC, GG was respectively 61%, 35% and 4% in patients and respectively 67%, 31% and 2% in the control group. The frequency of G and C alleles was respectively 78.5% and 21.5% in the patient group, and respectively 82.5% and 17.5% in control group. No statistically significant difference was observed between the two groups in frequency of genotypes and alleles.

**Conclusion:** This study showed no correlation between IL-6-174 G/C gene polymorphism and chronic periodontitis.

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### Introduction

Chronic periodontitis is a multifactorial disease that causes rapid destruction of the tissues supporting the teeth. This disease which begins with growth of gram-negative bacteria in periodontal pocket affects 10-15 percent of the adult population and is the main cause of tooth loss in them.

Cytokines are inflammatory mediators which contribute to the regulation of immune and inflammatory responses. These mediators play an important role in the initiation and expansion of inflammatory reaction and in periodontitis disease, lead to the progress of inflammation at presence of bacteria-derived pathogenic factors such as produced lipopolysaccharides, and cause destruction of periodontal tissues through producing prostaglandins and inducing collagenases and other proteases. Finally, the created inflammatory process leads to the disconnection of tissue, alveolar bone destruction and loss of teeth. IL-6 is a cytokine that has multiple functions such as differentiation or activation of macrophages and lymphocyte T, development and differentiation of lymphocyte B, stimulation of

hematopoietic process, stimulation of collagen synthesis and glycosaminoglycan, production of fibroblasts and proliferation of epithelial cells. It is also a potential stimulus for osteoclast differentiation, bone absorption and bone formation inhibition. This cytokine may act as a pre-inflammatory or anti-inflammatory and is one of the most important cytokines in the inflammatory reactions and is involved in the pathogenesis of several inflammatory diseases such as rheumatoid arthritis and psoriasis. In chronic periodontitis, increased gene expression in affected tissues has been reported compared to normal tissues. Thus, it is suggested that this cytokine is a diagnostic marker of periodontitis.

In addition to bacteria and other environmental factors that are involved in the onset of periodontitis, there are a lot of evidence about the role of genetic factors in the initiation and progression of this disease. A study conducted on 117 twin pairs revealed that chronic periodontitis is inherited in 50 percent of cases. IL-6 (-174G/C) promoter gene polymorphism can affect the gene expression. Several studies

indicate the impact of this polymorphism on chronic periodontitis. Babel et al in Germany investigated IL-6 (-174G/C) gene polymorphism in patients with chronic periodontitis and concluded that polymorphism of this gene may be associated with chronic periodontitis. However, in some other studies no relationship has been reported between this polymorphism and chronic periodontitis. Thus, this study examined the effect of IL-6 (-174G/C) gene polymorphism on incidence of chronic periodontitis in Iranian Sistan & Baluchestan population.

## Materials and Methods

This case- control study was conducted in Periodontology Department of Zahedan Dental School and Tropical and Infectious Diseases Research Center. Among the people referred to Periodontology department, 100 patients with chronic periodontitis as case group and 100 healthy subjects referred to Dental School whose health was confirmed in the periodontal examination and were similar to the patient group in terms of ethnicity and gender as control group, were studied.

The disease was diagnosed based on medical and dental history, radiographic findings (if necessary), reviewing clinical indicators, including tissue disconnection, bleeding during probing, tooth mobility, plaque and calculus. The average age of patients and healthy individuals was respectively  $35.82 \pm 1/18$  and  $28.78 \pm 9$ .

All subjects were generally healthy and had at least 20 teeth. Smokers, pregnant women and persons with systemic disorders such as diabetes, immunological disorders, hepatitis, viral infections, and long-term use of anti-inflammatory drugs, chemotherapy and orthodontic instruments were excluded. After getting approval from the University Ethics Committee (No. 89-3924) and written informed consent from the participants in the study, 2ml environmental blood was taken from them and was put into tubes containing EDTA. Then, it was transferred to the Tropical and Infectious Diseases Research Center and was stored at 20 °C until it was needed to be used.

DNA was extracted through Salting out method. To determine the type of polymorphism, Tetra amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) was performed by Thermocycler (Corbett- Australia) using specific primers (table 1 and figure 1).

PCR reactions were performed in micro tubes containing 25  $\mu$ l of reagents including: 50 ng of DNA sample, 250  $\mu$ M of dNTP, 1.5 mM mgcl<sub>2</sub>, 1 unit of Taq DNA polymerase and 0.4  $\mu$ M of each primer, under the following conditions: temperature 95°C for 5 minutes for initial denaturation, then 30 cycles

including: temperature of 95°C for 30 seconds for denaturation, temperature 56 C for 30 seconds for the connection of primers, 72°C for 40 seconds for extension and 72°C for 10 minutes for final extension.

After PCR, to ensure the accuracy of its performance, the PCR products were stained with ethidium bromide and electrophoresed within 3% agarose gel. Then bands were seen by UV ray (Gel-documentation, Germany).The difference between frequency of genotypes and alleles in patient and control groups was determined by chi-square test using SPSS-16 software.  $p < 0/05$  was considered significant. Logistic regression test was used to estimate the odds ratio (OR) and confidence interval 95% (CI).

## Results

Demographic data of patients and control group are presented in table 2. According to the information in this table, given that the patient and control groups were assimilated in terms of gender and ethnicity, there was no significant difference between the two groups in terms of gender and ethnicity distribution. The frequency of genotypes and alleles of IL-6 (-174G/C) gene polymorphism is shown in table 3. The frequency of genotypes GG GC, and CC of IL-6 (-174G/C) gene polymorphism was respectively 61%, 35% and 4% in patients, and respectively 67%, 31% and 2% in the control group and there was no significant difference between two groups.

The frequency of G allele was 78.5 percent in the patient group and 82.5% in the control group, and frequency of C allele was 21.5% in the patient group and 17.5% in the control group, and no significant difference was found between the two groups. With regard to age and gender, the odds ratio of mutant homozygous to normal homozygous (GG/CC) were calculated 2.82 (95% confidence intervals 0.48-16.82) and odds ratio of heterozygous to normal homozygous (GG/GC) 1.30 (95% confidence intervals 0.70-2.44).

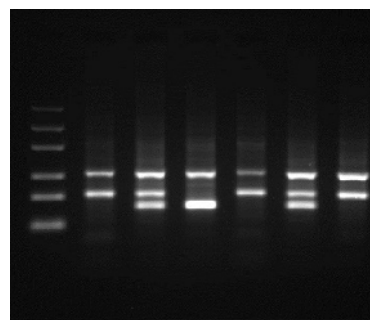


Figure 1. The primers sequences used for detection of polymorphism

**Table 1.** Primers of Interleukin-6 gene

Primers	Primer Sequence
Forward inner primer (G allele):	5'- TTTCCCCTAGTTGTGCTTCCG-3'
Reverse inner primer (C allele):	5'- TGCAATGTGACGTCCCTTAGCTTG-3'
Forward outer primer	5'- TGCAAGACATGCCAAAGTGCTG-3'
Reverse outer primer	5'- GGGCAGAATGAGCCTCAGACAT-3'

**Table 2.** Demographic parameters of chronic periodontitis and control groups.

Demographic parameters	Control group (n=100)	Chronic periodontitis group (n=100)	p- Value
Age(year)	28. 8±9	35.8±1.2	0.018
Gender (n%)			
Male	41(41)	43(43)	0.839
Female	59(59)	57(57)	
Ethnicity (n%)			
Sistani	45(45)	41(41)	0.282
Baluch	35(35)	40(40)	
Others	25(25)	19(19)	

**Table 3.** Genotypes and allele distribution of IL-6 (-174G/C) gene polymorphisms in chronic periodontitis (CP) patients and controls (C).

polymorphisms	CP (n%)	C (n%)	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Genotypes						
GG	61(61)	67(67)	1.00	Reference	1.00	Reference
GC	35(35)	31(31)	1.24(0.68-2.25)	0.478	1.30(2.44-0.7)	0.408
CC	4(4)	2(2)	2.19(0.39-12.42)	0.373	2.82(16.82-0.48)	0.253
Alleles						
G	157(78.5)	165(82.5)	1.00	Reference	1.00	
C	43(21.5)	35(17.5)	1.29(0.79-2.12)	0.3		

## Discussion

In this study, there was no relationship between IL-6-174G/C gene polymorphism and chronic periodontitis and the frequency distribution of genotypes and alleles showed no significant difference between subjects with chronic periodontitis and healthy subjects.

Chronic periodontitis is a multifactorial disease that the immune system plays an important role in creating and expanding of it. In recent years numerous studies have been conducted on the role of genes in the host response and progression of periodontitis, suggesting that most of the genes that are involved in the progression of periodontitis are associated with the immune system. Four IL-6 promoter gene polymorphisms of -174G/C, -572G/C, -373AnTn and -579G/C have been identified. The studies have shown that -174G/C gene polymorphism affects the expression and production of protein.

In 2002, Trevalatto et al revealed that there is a significant relationship between IL-6 (-174G/C) gene polymorphism and chronic periodontitis in Caucasian- Brazilian population and demonstrated that GG genotype will make the individual prone to the disease and the presence

of C allele compared to the GG genotype leads to the decrease in IL-6 gene expression after receiving an inflammatory stimulus, and has a protective role in disease progression.

In this study, frequency of G allele was reported 76% in patients group and 62/5% in control group. The protective role and effect of C allele on IL-6 protein production is likely, because IL-6 (-174G/C) gene polymorphism is located in the negative regulation domain (NRD) of IL-6 gene and can have a negative regulatory effect on its expression. In contrast to the results of Trevalatto, in this study, there is no relationship between this polymorphism and chronic periodontitis. In addition, G allele frequency showed no significant difference between the two groups. Raunio et al in Finland also reviewed the effect of IL-6 (-174G/C) gene polymorphism on the severity of periodontal disease in patients with type I diabetes and concluded that this polymorphism may affect the severity of periodontal disease. In addition, the probing depth and bleeding during probing in subjects with the GG genotype was higher than those with GC or CC.

In the similar study conducted in Brazil, Costa et al examined the impact of this polymorphism on patients with chronic periodontitis and revealed that the frequency of GG genotype in the case group is more than control group. Therefore, it is concluded that G allele may have an important role in the progression of periodontitis.

In the study conducted by Kalburgi et al in 2010, the obtained results were similar to the results of the above studies. In addition, the studies conducted in Egypt by Settin et al as well as the other study conducted in Germany by Babel et al, IL-6 (-174G/C) gene polymorphism is associated with periodontitis disease, with this difference that they reported CC genotype as a risk factor and the frequency of this genotype in patients was more than healthy people. Unlike these studies which have shown a positive relationship between IL-6 polymorphism and chronic periodontitis, in the present study, the results were shown as the lack of relationship between this gene polymorphism and chronic periodontitis. However, there are studies consistent with the present study that confirms our results.

The study conducted by Komatso et al showed no relationship between IL-6 (-174G/C) gene polymorphism and chronic periodontitis and all subjects had GG genotype. The studies conducted in Czechoslovakia and North America showed no relationship between this polymorphism and chronic periodontitis.

In a meta-analysis study conducted in 2009, Shao et al examined the six studies conducted on 1093 patients with chronic periodontitis and 574 healthy control group, and found no significant

correlation between IL-6 (-174G/C) gene polymorphisms and chronic periodontitis.

As shown in the mentioned studies, the obtained results vary in different populations. Racial and ethnic differences could be the reason of these inconsistencies. In addition, factors such as clinical diagnosis and environmental variables may also explain these differences. The studies concerning the relationship between interleukin-6 polymorphisms and other diseases can also be used to compare the genotype distribution in our control population and theirs.

The results of this study shows the relationship between IL-6 (-174G/C) gene polymorphisms and chronic periodontitis in Iranian-Sistani Baluchistan population and suggests that this gene cannot be the marker determining genetic predisposition to chronic periodontitis. However, further studies should be performed in this field in different regions, because understanding the genetic basis periodontitis diseases may be helpful in the diagnosis and treatment.

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#### References

1. Settin A, Abu-Seif M, Al shahat M, et al. Gene polymorphisms of TNF- $\alpha$  -308(G/A), IL-10-1082(G/A), IL-6-174(G/C) and IL1Ra (VNTR) in Egyptian cases with adult and early onset periodontitis. *Dent Sci* 2006; 4(1).
2. Costa AM, Guimaraes MCM, de Souza ER, et al. Interleukin-6(G-174C) and tumor necrosis factor-alpha (G-308A) gen polymorphism in geriatric patients with chronic periodontitis. *Gerodontology* 2010; 27(1): 70-75.
3. Babel N, Cherepnev G, Babel D, et al. Analysis of tumor necrosis factor-alpha, transforming growth factor- $\beta$ , interleukin-10, IL-6 and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J periodontol* 2006; 77(12): 1978-83.
4. Shao MY, Huang P, Cheng R and Hu T. Interleukin-6 gen polymorphisms modify the risk of periodontitis: A systemic review and meta-analysis. *J Zhejiang Univ Sci B* 2009; 10(12): 920- 927.
5. Dutra WO, Moreira PR, Souza PE, et al. Implication of cytokine gene polymorphisms on the orchestration of the immune response: Lessons learned from oral diseases. *Cytokine Growth Factor Rev* 2009; 20(3): 223-32.
6. Xiao LM, Yan YX, Xie CJ, et al. Association among interleukin-6 gene polymorphism, diabetes and periodontitis in a Chinese population. *Oral Dis* 2009; 15(8): 547-553.
7. Moreira PR, Lima PMA, Sathler KOB, et al. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. *Clin Exp Immunol* 2007; 148(1): 119-126

8. Irwin CR, Myrillas TT. The role of IL-6 in the pathogenesis of periodontal disease. *Oral Dis* 1998; 4(1): 43-7.
9. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nat Rev Cancer* 2008; 8(11): 887-899.
10. Grossman RM, Krueger J, Yourish D, et al. Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 1993; 86(16): 6367- 6371.
11. Houssiau FA, Devogelaer JP, Van Damme J, et al. Interleukin-6 in synovial fluid and serum of patients with rheumatoid and other inflammatory arthritides. *Arthritis Rheum* 1998; 31(6): 784-788.
12. Trevisatto PC, Scarel-Caminaga RM, De Brito RB Jr, et al. Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a caucasian Brazilian population. *J Clin Periodontol* 2003; 30(5): 438-442.
13. Kinane D, Hart TC. Genes and gene polymorphisms associated with periodontal disease. *Crit Rev Oral Biol Med* 2003; 14(6): 430-449.
14. Laine ML, Loos BG, Crielaard W. Gene Polymorphism in chronic periodontitis. *Int J Dent* 2010; 2010: 1-22.
15. Komatsu Y, Tai H, Galicia JC, et al. Interleukin-6 (IL-6) -373A9T11 allele is associated with reduced susceptibility to chronic periodontitis in Japanese subjects and decreased serum IL-6 level. *Dep Oral Biol Sci* 2005; 65(1): 110-114.
16. Holla LI, Fassmann A, Stejskalova A, et al. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients With chronic periodontitis. *J Periodontol* 2004; 75(1): 30-36.
17. Jingjin L, Zemin G, Xin M, et al. Correlation between an interleukin-6 -572C/G polymorphism and chronic periodontitis. *Int J Periodontol Rest Dent* 2010; 30(3): 301-305.
18. Raunio T, Knuutila M, Hiltunen L, et al. IL -6-174 genotype associated with extent of periodontal disease in Type1 diabetic subjects. *J Clin Periodontol* 2009; 36: 11-17.
19. Kalburgi N, Bhatia A, Bilichodmath S, et al. Interleukin-6 promoter polymorphism (-174G/C) in indian patients with chronic periodontitis. *J Oral Sci* 2010; 52(3): 431-437.
20. Wohlfahrt JC, Wu T, Hodges J S, et al. No association between selected candidate gene polymorphisms and severe chronic periodontitis. *J Periodontol* 2006; 77(3): 426-436.
21. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphism in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998; 102(7): 1369-1376.
22. Stabholz A, Soskolne W A, Shapira L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *J Periodontol* 2000; 53: 138-153.

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