

# Study of IL-10, IL-18 Polymorphisms in the Sulfur Agent Patients with and without Periodontitis

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## Abstract

**Background and aims.** Periodontitis is a multifactorial disease. Varieties of microbial and environmental, as well as genetic factors are reported to influence risk for periodontitis. The present study sought to test the putative involvement of IL-10 and IL-18 gene polymorphism in pre-disposition to periodontitis in patients had exposure to chemical agent as a risk factor for periodontitis development.

**Materials and methods.** In this retrospective cohort study, 82-chemical-injured patients selected according to inclusion criteria (lack of disease in soft and hard oral tissue, excluding dental caries or periodontal disease, free of orthodontic appliances, not received drug, non-smoker, and lack if systemic disease were examined with use of periodontitis clinical criteria (clinical attachment loss, probing depth, bleeding on probing) at periodontology department, dental clinic. Patients were assigned into two groups with periodontitis and without periodontitis. A blood sample (10 ml) of each participant was tested by use of polymerase and IL-10 and IL-18 polymorphism determined. Data were statistically analyzed using t-test by SPSS software to detect polymorphism and genetic differences between two groups.

**Results.** The study showed that in patients with periodontitis A allele (64.4%) was occurred more frequently than G allele (35.6%) at position -1082 of the IL-10 gene, and susceptibility to periodontitis increases with presentation of A allele ( $P<0.05$ ). In patients with periodontitis, C allele (57.7%) was more frequent than T allele (42.3%), and in healthy subjects, T allele (55%) was more frequent than C allele (45%) at position 819 of IL-10 gene. However, these differences were not significant ( $P<0.05$ ). IL-18 gene polymorphisms were not statistically different between two groups.

**Conclusion.** The study concluded that in chemical-injured subjects -1082 polymorphisms of the IL-10 gene were associated with periodontitis. IL-18 gene polymorphisms were not associated with destructive periodontal disease, but -819 polymorphisms of the IL-10 gene and IL-18 gene polymorphisms not associated. Exposure to chemical agents may affect pre-disposition to periodontitis development.

**Key words:** IL-10, IL-18, Periodontitis, Polymorphisms.

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## Introduction

Periodontal diseases start with collection of microbial plaque into gingival clefts and the activities of these microbes make inflammation at these points. This primary inflammation is known as gingivitis may progress to chronic destructive inflammatory diseases which is known as periodontitis. Gingivitis is a reversible process however, periodontitis is an irreversible process that is accompanied with destruction of bones (at the site of teeth) and the other supporting tissues of the teeth. Periodontitis is a multifactorial disease. A variety of different factors like microbial and environmental and host defense responses are reported to influence risk for periodontitis and it was sought the severity of all of these factors are under the genetic factors.

In the war between of Iran and Iraq many injured remained that some of them are under the problems of chemical agents like destructive chemical gases. One of the most chemical gases was used in this war was Khardal (Phosphogen gas). Toxicity problems with phosphogen agent are classified into two groups: Alkilation of important intracellular molecules like DNA and proteins and another is contact (direct) effects. Alkilation of DNA makes changes in the scheme of the molecule and stops the Mitosis of genetic materials. The most tissues that injured from this process are immune system, skin and gastrointestinal system that have high rate division.

According to our clinical experience, most of the chemical-agent patient suffered from periodontitis. According to high frequency of the disease among the chemical-agent patients there is a possibility that injured with chemical agent may has putative influence on increasing the incidence and progression of periodontitis. In the late past years, many efforts have been done for identification of Allele diversity of genes has responsibility for periodontitis. Then if the genetic basis of periodontitis has been known can perform important information about the diagnosis and treatment of the disease. Nevertheless, there is a little report that focuses on the identification of genes polymorphism that has clinical importance.<sup>1,2</sup> Then, in this study, we try to find the relationship between IL-10 and IL-18 gene polymorphisms with incidence of periodontitis among chemical-injured patients. The results of this study and the studies like that can make new progress in the diagnosis and treatment of diseases like gene therapy.

According to the literature in this filed, there have been many not integrated efforts which could be

mentioned.<sup>3-24</sup> However, the following studies are the most related ones in this respect:

Yamazaki and et al<sup>24</sup> extracted the DNA of peripheral blood cells from a Japanese population contain 34 patients with adult periodontitis, 18 patient with generalized periodontitis and 52 healthy individuals and identify the IL-10 genotype in 506 and 1140 regions. Comparison of allele frequency and haplotype between the patients and healthy individuals has showed no significant and statistically meaningful difference.

Gonzales et al<sup>25</sup> identify a German population contain 21 patients with chronic periodontitis and 18 patients with invasive periodontitis according to clinical and radiographic parameters and IL-10 gene polymorphism of these patients has been compared with 21 healthy individuals with same age. All genotypes have been identified with PCR method and restriction enzyme cleavage in 824 and 597 regions. The analysis showed that the frequency of alleles among the patients and healthy individuals has no significant and meaningful difference. According to this study, the final result has been shown that there is no relationship between the polymorphisms and periodontal diseases.

Breglndh et al<sup>26</sup> evaluated the IL-10 gene polymorphisms in 1087 among Swedish individuals contain 60 patients with severe chronic generalized periodontitis and 31 healthy individuals with same age. Genotypes were identified by DNA extracted from peripheral blood cells, and compared between two groups. The results have showed the percentage of individuals with genotype GG in patient group are significantly more than healthy individuals. This study suggests there is a relationship between the IL-10 gene polymorphism in 1087 region in Caucasian of North-Europe with sever chronic periodontitis incidence.

Scarel-Caminaga et al<sup>27</sup> in Brazil extracted and analyzed the DNA of 67 with chronic periodontitis and 43 healthy individuals (all of them were non-smoker) by PCR method. They evaluated IL-10 gene in 1087, 819 and 592 regions and compared them between two groups. The results of study have showed the frequency of 819 and 592 regions polymorphisms had significantly difference between two groups. In addition, it has concluded that there was a relationship between haplotype ATA and the severity of disease among women with chronic periodontitis.

Loos et al<sup>28</sup> after reviewing the past studies have found some polymorphisms in genes coding HL-1,  $\gamma$  receptors and Vit D receptors may have relationship with incidence of chronic periodontitis in some races. They found out this relationship different between different populations.

Babel et al<sup>29</sup> identified the genotype of 122 German individuals with chronic periodontitis and 114 healthy individuals with same age with PCR-SSP method. According to findings of this study IL-10 polymorphism didn't have significant and meaningful difference between two groups.

Mellati et al<sup>30</sup> have studied the relationship between IL-10 gene polymorphism in 1082 region and invasive generalized periodontitis. In this study, 52 of Iranian individuals from Khorasan with periodontitis have been compared with 61 healthy individuals with same gender, age and race. The genotype of all participants has identified by extraction of DNA and usage of ARMS-PCR method. The results have showed no significant and meaningful difference between the patients and healthy individuals. In addition, there was no significant relationship between periodontal diseases and alleles frequency among these groups. This study has showed nucleotide A polymorphism in 108 region of IL-10 gene hasn't significant relationship with incidence of invasive generalized periodontitis.

Trevonen et al<sup>31</sup> has studied the relationship between IL-10 polymorphism and periodontitis with PCR method in Finland individuals. In this study, the frequency of genotype in 51 patients has compared with 178 healthy individuals without periodontal diseases and has concluded there was no significant and meaningful relationship between IL-10 genotype frequencies among healthy individuals and patients.

Sumer et al<sup>32</sup> has studied the relationship between IL-10 gene polymorphism and chronic generalized periodontitis in Turkish population. DNA samples of 57 patients and 73 healthy individuals have been collected and the polymorphisms have been identified with REC method. The results have showed there was a significant and meaningful relationship about alleles and genotypes frequencies in region 597 between patients and healthy individuals, but there was no relationship with 824 region polymorphism. According to this study, it has concluded that there is a significant relationship between IL-10 gene polymorphism in 597 region and severe chronic generalized periodontitis.

Reichert et al<sup>33</sup> has studied IL-10 gene polymorphism in 1082, 819 and 819 regions in German population contain 27 patients with chronic generalized patients and 32 patients with invasive generalized periodontitis and compared them to 32 healthy individuals without any periodontal problems. IL-10 polymorphisms have been analyzed with PCR-SSP method. According to the results of this study, the ATA/ATA genotype has been seen just in patients with invasive periodontitis and has been concluded that ATA haplotype accompanies with low production of IL-10 and has been considered as a major risk factor for invasive generalized periodontitis. In addition, it has been seen that amount of *Prevotella Intermedia* was low in individuals with ACC, ATA and ACC/ATA and was high in individuals with GCC/GCC genotype.

Flowaczny et al<sup>34</sup> has studied the relationship of IL-10 gene different polymorphisms and incidence of destructive periodontal diseases among German individuals. In this study IL-18 gene polymorphism genotype in 656, 607, 137, 113, and 127 regions and codon 35/3 in 123 patients with periodontitis and 121 healthy individuals has been identified and analyzed. The results have showed that the genotype frequencies for IL-18 polymorphisms in every 6 regions have no differences between two groups. In addition, it has been concluded that haplotype distribution of two alleles of 607 and 137 regions have no differences between two groups. According to this study IL-18 gene polymorphism in every 6 regions have no relationship with destructive periodontitis.

### **Materials and Methods**

This study is a retrospective cohort study. All of the chemical-injured patients of Hamadan have been classified in two groups according to clinical parameters; patients with periodontitis and patient without periodontitis.

In this case control study chemical-injured patients of Hamadan has been invited to periodontics ward of dentistry faculty of Hamadan and after explanation of aims and steps of study for them and taking informed consent, has been entered to study under inclusion criteria (without hard or soft mouth tissue diseases except caries and periodontal diseases, no usage of orthodontic equipment, no drug usage, no smoking and no systemic diseases history). All of the participants has been evaluated for periodontitis clinical diagnostic criteria (clinical attachment loss, probing depth, bleeding on probing, plaque control record) and classified into two groups; a group with perio-

dontitis and a group without periodontitis. 10 ml venous blood sample was obtained from each participant and IL-10 and IL-18 genotype has been identified by using the special kits and polymerase chain reaction method.

**Results**

In this study, 88 chemical-injured men was evaluated for periodontitis. Six patients were excluded because of systemic disease and smoking and 82 patients was entered into study. According to diagnostic criteria of periodontitis (PD, CAL, PCI and BPI) 52 patients (63.4%) was diagnosed with periodontitis and 30 patients didn't have periodontitis. the mean age in case group was 48.6±6.1 and 46.3±7.5 years in control group (Table1).

**Table 1.** Distribution of age frequency of chemical-injured patients according to periodontitis

Group	Standard Deviation	Mean	No (%)	P-value
With periodontitis	6.1	4.6	52 (63.4)	0.860
Without Periodontitis	7.5	46.3	30 (36.6)	

P<0.05

Frequency of allele A in region G/A 1082 was 64.4% in case group and 21.7% in control group. Significant difference was detected between two groups (P=0.000). Frequency of allele G was 35.6% in case group and 78.3% in control group. Significant difference was detected between two groups (P=0.001). Frequency of genotype AA, AG and GG was 42.3%, 44.2% and 13.5% respectively in case group and 10%, 23.3% and 66.7% in control group. No significant difference was detected between two groups Table2).

Frequency of allele C in region 819 was 57.7% and 45% in case and control groups respectively. No significant difference was detected between two groups (P>0.05). Frequency of allele T was 42.3% and 55% in case and control groups respectively. No significant difference was detected between two groups (P>0.05). Frequency of genotype CC, CT and TT was 27%, 61.5% and 11% in case group respectively and 16.7%, 56.7% and 26.6% in control group respectively (Table3). No significant difference was detected between two groups (P>0.05).

**Table 2.** Distribution of IL-10 alleles and genes frequencies in chemical-injured with and without periodontitis

IL-10-1082 Polymorphisms	With periodontitis	Without periodontitis	P-Value
Alleles			
A	67 (64.4%)	13 (21.7%)	0.000
G	37 (35.6%)	47 (78.3%)	0.001
Genotypes			
AA	22 (42.3%)	3 (10%)	0.000
AG	23 (44.2%)	7 (23.3%)	
GG	7 (13.5%)	20 (66.7%)	

P<0.05

**Table 3.** Distribution of IL-10 alleles and genes frequencies in chemical-injured with and without periodontitis

IL-10-819 Polymorphisms	With periodontitis	Without periodontitis	P-Value
Alleles			
C	60 (57.7%)	27 (45%)	0.830
T	44 (42.3%)	33 (55%)	0.289
Genotypes			
CC	14 (27%)	5 (16.7%)	0.175
CT	32 (61.5%)	17 (56.7%)	
TT	6 (11.5%)	8 (26.6%)	

P<0.05

**Table 4.** Distribution of IL-18 alleles and genes frequencies in chemical-injured with and without periodontitis

IL-18 Polymorphisms	With periodontitis	Without periodontitis	P-Value
Alleles			
F1	70 (63.3%)	38 (63.3%)	0.800
F2	34 (32.7%)	22 (36.7%)	0.359
Genotypes			
F1F1	20 (38.5%)	12 (40%)	0.492
F1F2	25 (48.1%)	14 (46.7%)	
F2F2	7 (13.4%)	4 (13.3%)	

P<0.05

According to Table 4, the IL-18 gene mononucleotide polymorphism, the frequency of allele F<sub>1</sub> was 67.3% and 63.3% in case and control group respectively. No significant difference was detected between two groups (P>0.05). The frequency of allele F<sub>2</sub> was 32.7% and 36.7% in case and control group respectively. No significant difference was detected between two groups (P>0.05).

Frequency of genotype F<sub>1</sub>F<sub>1</sub>, F<sub>1</sub>F<sub>2</sub> and F<sub>2</sub>F<sub>2</sub> was 38.5%, 48.1% and 13.4% in case group respectively and 40%, 46.7% and 13.3% in control group respec-

tively. No significant difference was detected between two groups ( $P>0.05$ ).

### Discussion

In this study, 82 male chemical-injured patients evaluated for periodontitis. According to periodontitis clinical diagnostic criteria (PD, CAL, PCI and BPI) 52 chemical-injured patients (63.4%) had periodontitis as a case group and 30 without periodontitis as a control group. The mean age was  $48.6\pm 6.1$  and  $46.3\pm 7.5$  years in case and control group respectively. No significant difference was detected between two groups ( $P>0.05$ ). Therefore, age was not an effective factor on pattern of incidence and progression of periodontitis in two groups and there were other factors that influenced on incidence of periodontitis.

Analysis of IL-10 G/A -1082 region mononucleotide polymorphism showed the frequency of A allele was 64.4% and 21.7% in case and control group respectively. Meaningful significant difference was detected between two groups in frequency of A allele ( $P=0.0000$ ). Frequency of allele G was 35.6% and 78.3% in case and control group respectively. Significant difference was detected between two groups in frequency of allele G ( $P=0.001$ ). This finding showed allele A significantly increases periodontitis. Frequency of genotype AA, AG and GG was 42.3%, 44.2% and 13.5% in case group respectively and 10%, 23.3% and 66.7% in control group respectively. Genotype AA and GG had same frequencies and were more than in patient group. Significant difference was detected between two groups in frequency of genotypes ( $P=0.000$ ). This finding showed that genotype AA and AG because of allele A increases the incidence of periodontitis.

Analysis of IL-10 C/T -819 region mononucleotide polymorphism showed the frequency of allele C was 57.7% and 45% in case and control group respectively. No significant difference was detected between two groups in frequency of C allele ( $P>0.05$ ). Frequency of allele T was 42.3% and 55% in case and control group respectively. No significant difference was detected between two groups in frequency of allele T ( $P>0.05$ ). Frequency of genotype CC, CT and TT was 27%, 61.5% and 11.5% in case group respectively and 16.7%, 56.7% and 26.6% in control group respectively. No Significant difference was detected between two groups in frequency of genotypes ( $P>0.05$ ). Genotype CT was more than genotype CC and TT in two groups. Comparison of two groups showed no significant relationship between IL-10 -

819 gene genotype and incidence of periodontitis.

Analysis of IL-18 mononucleotide polymorphism showed the frequency of allele  $F_1$  was 67.3% and 63.3% in case and control group respectively. No significant difference was detected between two groups in frequency of  $F_1$  allele ( $P>0.05$ ). Frequency of allele  $F_2$  was 32.7% and 36.7% in case and control group respectively. No significant difference was detected between two groups in frequency of allele T ( $P>0.05$ ). Frequency of genotype  $F_1F_1$ ,  $F_1F_2$  and  $F_2F_2$  was 38.5%, 48.1% and 13.4% in case group respectively and 40%, 46.7% and 13.3% in control group respectively. No Significant difference was detected between two groups in frequency of genotypes ( $P>0.05$ ). Frequency of allele  $F_1$  was more than  $F_2$  in two groups. The mot genotype was  $F_1F_2$  in two groups. No significant difference was detected between two groups in alleles and genotypes. Therefore, there was no significant relationship between IL-18 gene polymorphism and incidence of periodontitis.

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