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Association between TNF-α (– 308 G → A) Gene Polymorphism and Chronic Periodontitis

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Abstract

***Background*:** Chronic periodontitis is an inflammatory disease caused by environmental and genetic factors. It leads to destruction of tooth supporting tissues and may cause tooth loss. Cytokine TNF-α plays a role in the development of inflammatory lesions and development and progression of the chronic periodontitis disease. Some polymorphisms of this gene are accompanied with change in expression level. The purpose of this study was to investigate the relationship between TNF-α -308 G>A (rs1800629) polymorphism and chronic periodontitis.

***Materials and Methods*:** In this case-control study, 100 patients with chronic periodontitis and 100 normal subjects, referring to the clinic of Zahedan Dental School, were evaluated. Venous blood samples of participants were taken. DNA was extracted using salting-out technique and gene polymorphism was studied at this position using specific primers by T-ARMS PCR method. To investigate the frequency of genotypes and alleles in both groups, $\chi 2$ test was employed and $p<0.05$ was taken to be statistically significant.

***Results*:** The frequencies of AA, GA, and GG genotypes in the patient and control groups were, 1%, 8%, and 91%, and 0%, 16%, and 85%. The frequencies of A and G allele in patient and control groups were 5% and 95%, and 7.9% and 92.1% respectively. There was no significant difference in the frequencies of genotypes and alleles between the groups.

***Conclusion*:** The present study indicates that there is no association between TNF-α (-308 G>A) polymorphism and chronic periodontitis in this population.

Introduction

Periodontitis is a polymicrobial chronic disease with multifactorial inheritance characterized by interaction between gram-negative bacteria and host inflammatory response [1, 2]. Destruction of tooth supporting tissues, tooth mobility, and tooth loss are considered to be among the most important symptoms of this disease [3]. The first etiologic factor in the periodontal diseases is the accumulation of bacteria in gingival groove [4]. The dental plaque bacteria and calculus have direct and indirect roles in the destruction of periodontal tissues. The direct impact is proteolytic enzymes secretion and immunosuppressive factors development. In indirect impact, bacteria-derived pathogenic factors such as lipopolysaccharides stimulate host immune response, leading to stimulation of proinflammatory cytokines synthesis, connective tissue breakdown, and bone resorption [5].

Cytokines are peptide mediators that interfere in regulating immunologic, inflammatory, systematic, and restorative responses against invading agents by stimulation of the proliferation and differentiation of the cells, and/or inhabitation of cells differentiation and proliferation [5]. Cytokine TNF-α is the most important response of the host against the active component of Gram-negative bacteria (endotoxin). It is mostly generated by mononuclear phagocytes, T lymphocytes, natural killer cells (NK), and activated mast cells [6]. By destructing arachidonic acid, this cytokine causes increment of prostaglandin E2 concentration, and then activation of osteoclasts. Consequently, along with IL-1, this causes bone resorption, resulting in release of the Matrix metalloproteinase (MMPs) and destruction of the extracellular matrix [7-9].

Cytokine TNF-α has significant proinflammatory activity and causes increased activity of connective tissue and endothelial cells [1]. Its activity is regulated by IL-10 and other anti-inflammatory cytokines. The balance between these cytokines is achieved by immunity system. Therefore, in inflammatory diseases superiority of proinflammatory cytokines is obvious [2]. Of the several cytokines activity regulating factors, gene polymorphism is more salient [10]. The presence of TNF-α gene

polymorphism is effective in cytokine gene expression and has important role in genetic regulation of the inflammatory responses, resistance, and/or susceptibility to inflammatory diseases [2].

TNF-α gene is located on the long arm of chromosome 6 within the MHC class III region. After determination of the sequence of the human TNF-α gene promoter region in different people by Wilson, a single nucleotide polymorphism was expressed at region -308, called TNF2 polymorphism. Then, the effect of this polymorphism on the expression of TNF-α gene was studied [4-7].

Changing G→A within TNF-α (-308) promoter region generates sequence to connect AP_2 translation factor which is associated with increased TNF-α production and translation as a strong inducer of tissue destruction and bone resorption in different varieties of periodontal diseases [8].

Some researchers have proven that the presence of allele A within TNF-α (-308) gene is associated with increased production of cytokine in patients with chronic periodontitis and swelled disease severity; while, in some other studies no relationship has been reported between this polymorphism and chronic periodontitis. The aim of this study is to investigate the relationship between TNF-α (-308 G>A) gene polymorphism and chronic periodontal disease in Iranian population of Sistan-and-Balouchistan, Zahedan.

Materials and Methods

This case-control study was conducted in Periodontology Department of Dental School and Infectious and Tropical diseases Research Center, of Zahedan University of Medical Sciences (ZUMS), Zahedan, Iran. The diagnosis of chronic periodontal disease was carried out by a periodontologist based on clinical examinations, medical and dental history, evaluating probing attachment loss and loosening of tooth, bleeding on probing (BOP), probe depth, and dental radiography.

Exclusion criteria were; taking anti-inflammatory drugs, using orthodontic instruments, smoking, pregnancy, oral cavity infections, diabetes types I and II, rheumatoid arthritis, lupus erythematosus, hepatitis, HIV, cardiovascular disease, and other systematic diseases with influence on periodontal condition.

All patients had at least 20 teeth. In this regard, 100 people (43 men and 57 women with mean age of 35.82±1.18 years) were put into chronic periodontitis group. A number of 100 patients who were referring to the clinic, had no symptoms of chronic periodontitis disease based on periodontal examinations, and were eligible for inclusion into the study were located in control group (41 men and 59 women with mean age of 28.78± 9.0). The samples were collected from the same geographical region in 6 months (October, 2010 to April, 2011).

The groups were matched based on sex and ethnicity. After getting approval from the University Ethics Committee (No. 89-3925) and written informed consent from the participants in the study, 2 ml 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 20ºC until it was needed to be used.

DNA was extracted using salting out technique. TNF-α rs1800629 polymorphism was evaluated by Tetra amplification Refractory Mutation System- Polymerase Chain Reaction (T-ARMS-PCR), which is a rapid, easy, and sensitive method. Specific T-ARMS-PCR primers were designed for investigating TNF-α polymorphism at position -30 G→A. The sequence of used primers is presented is Table 1.

For performing PCR, PreMix prepared microtubes (Bioneer Co.) were employed. One µl DNA, 0.7 µl inner primers, and 0.5 µl outer primers (10 mM), 1.5 µl DMSO, and 16 mµl sterile distilled water were added to the microtube.

PCR was carried out using thermocycler (Corbett, Australia) with initial denaturation at 95ºC for 5 minutes. Then 30 cycles including: temperature of 95ºC for 30 seconds for denaturation, temperature 68ºC for 20 seconds for the connection of primers, 72ºC for 15 seconds for extension and 72ºC for 10 minutes for final extension.

The PCR products were analyzed by electrophoresis on 2% agarose gels containing 0.5 µg/ml ethidium bromide. Then the bands were seen under UV ray (Gel-documentation, Germany) figure 1.

To compare frequency of genotypes and alleles in normal and patient groups, χ^2 (SPSS-16) was used, and $p<0.05$ was considered as statistically significant. Logistic regression test was used to estimate the odds ratio (OR) and confidence interval 95% (CI).

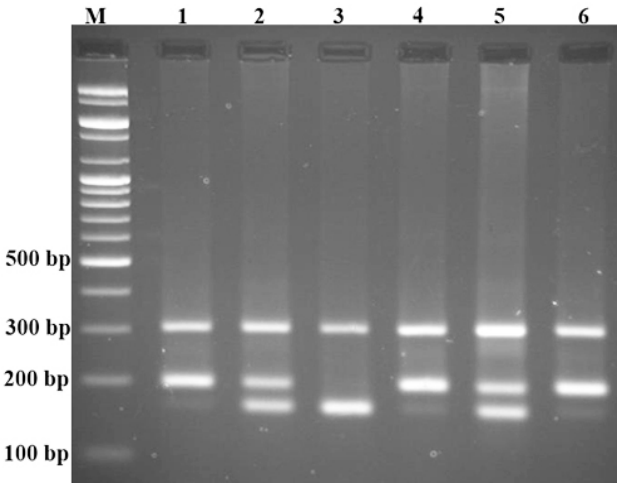

Figure 1. Electrophoretic scheme of T-ARMS-PCR generated bands for determination of TNF-α 308 G>A gene polymorphism.
Left to right: M, DNA marker, wells #1, 4, and 6: GG homozygous (304 bp and 197 bp), wells #2 and 5: heterozygous GA (304 bo, 197 bp, and 162 bp), well #3: homozygous AA (304 bp and 162 bp)

Table 1. Sequence of the primers used for TNF-α polymorphism determination

Tm (ºC)	Primer Sequence	Primers
65.3	5′-GGAGGCAATAGGTTTTGAGGCGCAGGG-3′	Forward inner primer (G allele)
67.1	5′-GTAGGACCCTGGAGGCTGAACCCCGTACT-3′	Reverse inner primer (A allele)
67.5	5′-AGGACTCAGCTTTCCGAAGCCCCTCCCA-3′	Forward outer primer
63.2	5′-TTCTGTCTCGGTTTCTTCTCCATCGCGG-3′	Reverse outer primer

Table 2. Demographic parameters of chronic periodontitis patients and control group

	Chronic periodontitis group	Control group	*p*-Value
Age(year)	35.8±1.2	28. 8±9	0.018
Gender (N)			
male	43	41	0.839
female	57	59	
Ethnicity (N)			
Sistani	41	45	0.282
Baluch	40	30	
Others	19	25	

Table 3. The frequency of genotypes and alleles of TNF-α (-308 G>A, rs1800629) polymorphism gene in 100 patients and 100 healthy subject

rs1800629 Polymorphisms	Chronic periodontitis N (%)	Control N (%)	*OR (95% CI)	*p*-Value
GG	91 (91.0)	84 (84.2)	Reference	-
GA	8 (8.0)	16 (15.8)	0.47 (0.19-1.15)	0.127
AA	1 (1.0)	0 (0.0)	-	-
GA+AA	9 (9.0)	16 (15.8)	0.48 (0.19-1.22)	0.125
Alleles				
G	190 (95)	186 (92.1)		
A	10 (5)	16 (7.9)	0.61 (0.27-1.38)	0.310

* Adjusted for age and sex

Results

Demographic characteristics of case and control groups are presented in table 2. Since both groups were matched for gender and ethnicity, no significant difference was observed between two groups in this regard.

The frequencies of genotypes and alleles of TNF-α (-308 A>G) polymorphism in both groups were compared in table 3. The frequencies of AA, GA, and GG genotypes in the patient and control groups were respectively, 1%, 8%, and 91%, and 0%, 16%, and 84%, indicating no significant difference in distribution of genotypes.

The frequencies of A allele in patient and control groups were 5% and 7.9% respectively. Therefore, no significant correlation between chronic periodontitis and A allele was observed. The frequencies of G allele in patient and control groups were 95% and 92.1% respectively, indicating no significant difference between the groups regarding G allele ($p>0.05$).

Given the sex and age, the odds ratio of heterozygous versus normal homozygous (GG/GA) was computed (1.30, 95% confidence interval=0.19-1.22).

Discussion

In the present study, no statistical significant difference was seen between the chronic periodontitis patient and normal subjects with respect to genotypes of TNF-α-308 G>A polymorphism. The frequency of A allele in patient and control groups were 5% and 7.9%, indicating no significant correlation between allele and chronic periodontitis disease.

Chronic periodontitis is an infectious disease, preliminary caused by bacteria existing in gingival margin. Then, by means of osteoclasts, proinflammatory cytokines directly stimulate bone resorption and increase MMPs secretion. This causes destruction of gingival extracellular matrix, periodontal ligament, and alveolar bone, and so is the main cause of tooth loss in adults. Therefore, so much secretion of TNF-α and IL-1b is related to the intensity of inflammatory lesions in periodontal patients [13].

Several genetic, environmental, ethnical, and sexual factors as well as the type of periodontal bacteria can affect disease process. There are strong evidences indicating greater impact of genetic factors than environmental ones on periodontitis development [14].

TNF-α (-308 A>G) gene polymorphism affects the expression of this cytokine. In addition, the presence of A allele in this region is accompanied by increased level of TNF-α and intensified periodontal disease [2, 11].

In a study by Settin et al., in 2006, on TNF-α (-308 A>G) polymorphism within an Egyptian population, greater frequency of GG homozygous genotype was observed in the patient group compared with the control group [14].

Abraham and Kroeger in a study have demonstrated the effect of A allele in -308 region on immune responses that make the person susceptible to infections and autoimmune diseases [15]. Kornman and di Giovine observed 38% frequency of A allele in TNF-α-308 region in patients with moderate periodontitis. In his study, the frequency of A allele in patient with severe periodontitis was 21 percent [16].

In a study conducted in 1998, Galbraith et al. reported 19% frequency of A allele in TNF-α-308 region in patients, while in 1999 they reported 3% frequency in the patients with advanced periodontitis [10].

Menezes et al. in a study on a Brazilian population showed that the frequency of A allele in control groups was greater than periodontitis group. However, statistically no significant difference was observed between them [8].

In a study by Flowaczny et al., the frequency of allele between patient and normal subjects was similar, and there was no correlation between this polymorphism and periodontitis disease [6]. Therefore, the frequency of A allele extensively differs in various populations (3-32%) [8]. Different types of polymorphisms in TNF-α promoter region in various ethnics are investigable [6]. This is probable that ethnical differences cause different frequencies in TNF-α genotypes [3].

Given rare alleles, limited sample size affects the relationship between clinical symptoms and genotypes [8]. Hence, for the first time, the relationship between A allele and development of periodontitis can be verified by increasing the sample size.

Some bacterial pathogens such as *T. forsythia* and *T. denticola* in in-vivo and in-vitro environments increase TNF-α level [11]. Therefore, the type of periodontal bacteria is effective both in TNF-α secretion and in periodontitis severity. Consequently, regarding multifactorial characteristics of this disease, the microbiological application is also recommended in future studies.

Fassmann et al. has suggested that the combination of TNF-α and TNF-β polymorphisms may affect individual's susceptibility to periodontitis disease. That is, gene-gene interaction in different polymorphisms is indicated [7]. It seems that investigation of single nucleotide polymorphism in TNF-α in this study has not been sufficient to clarify the genetic background of chronic periodontitis. For that reason, studying other polymorphisms of this gene and other genes are required.

Certainly, the subjects in control group were not genetically periodontitis-resistant, so it is only possible to control etiologic factors of this disease with oral hygiene examination, in which periodontal pathogenic process is considerably slow [11].

The results of this study indicate that TNF-α-308 G>A polymorphism is not a chronic periodontitis risk factor in this study population, However, further studies should be performed in this field.

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Authors' Contributions

Hamid Mahmoudzadeh-Sagheb and Zahra Heidari conceived and co-designed the study, supervised all the experimental design, analyzed the results, and drafted the manuscript. Somaieh Solhjoo carried out the sampling, DNA extraction and genotyping analysis and participated in drafting the manuscript. Mohammad Hashemi contributed in study design and coordination, supervised the genetic assays and statistical analysis and the interpretation of the results. Mohammadayub Rigi-Ladez performed patient selection and participated in data collection. All authors read, modified and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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