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Gene Polymorphisms of TNF-α and IL-1β Are Not Associated with Generalized Aggressive Periodontitis in an Iranian Subpopulation

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ABSTRACT

Cytokines play a part in pathogenesis of periodontitis via inflammation phenomenon. Aggressive periodontitis (AgP) is a multifactorial disease resulting in rapid tooth loss due to severe destruction of tooth supporting apparatus. Recently, researchers have focused on genetic susceptibility of periodontitis through investigating the gene variations of cytokines and other components of immune response. In this study we analyzed single nucleotide polymorphism (SNP) of two cytokines in association with AgP in an Iranian-Khorasani population; Interleukin-1 beta (IL-1β) +3954 C/T and Tumor Necrosis Factor alpha (TNF-α) -308 G/A.

From arm vein of patients (n=58) and periodontally healthy individuals (n=60) blood sample was obtained and the DNA was extracted. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) procedure was performed to recognize the SNPs. X^2 test was used to determine the statistically significant differences between the two groups.

The frequency of genotypes and alleles had no significant differences between patients and control groups. The distributions were as follows. IL-1β +3954: CT, CC and TT genotypes in patients were 39.6%, 60.4% and 0.0% and in controls were 41.7%, 50% and 8.3%, respectively. TNF-α -308: GA, GG and AA genotypes in patients were 44.8%, 41.4% and 13.8% and in controls were 46.7%, 50% and 3.3%, respectively.

This investigation do not substantiates the role of IL-1β +3954 and TNF-α -308 polymorphisms, separately, as risk determinants for AgP in Iranian population. Further research based on all components of immune response, is needed to corroborate the genetic susceptibility of AgP.

Keywords: Aggressive periodontitis, Gene polymorphism, Interleukin-1 beta, Tumor Necrosis Factor alpha

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INTRODUCTION

Periodontitis is an inflammatory disease of supporting tissues of the teeth and is initiated by groups of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone. Aggressive periodontitis differs from chronic periodontitis (ChP) primarily by the rapid rate of disease progression, being seen in an otherwise healthy individual, an absence of large accumulation of plaque and calculus, and a genetic familial trait.[1,2]

Cytokine network, a crucial aspect of host inflammatory response, has attracted much attention as potentially important parameter in periodontitis. The concentration of cytokines has been higher in gingival fluid and periodontium of individuals suffering from periodontitis.[3-7] Proinflammatory cytokines promote synthesis of endothelium adhesion molecules on surface of inflammatory cells leading to vasodilatation, chemotaxis and inflammation.[8,9]

Interleukin-1(IL-1), a Proinflammatory cytokine, increases production of antibodies and cytokines in lymphocytes, stimulates secretion of collagenase and prostaglandin E2 and results in inflammation and bone loss in periodontium and other tissue organs.[8,10] IL-1 is produced in three forms; IL-1β, IL-1α and IL-1 receptor antagonist (IL-1RN). IL-1RN regulates the inflammatory function of IL-1α and IL-1β.

Secretion of TNF-α is one of the most important host responses to endotoxin of gram negative microbes. TNF-α activates chemotaxis of inflammatory cells, stimulates production of IL-1, IL-6 and IL-8, increases function of lymphocytes and has a role in creating fever, regulating coagulation and inhibiting proliferation of stem cells in bone marrow.[2,11]

Single nucleotide polymorphisms (SNPs) in genes encoding cytokines may bring about significant effects on their production and function. Therefore, SNPs may theoretically play a role in pathogenesis of periodontitis by changing the level and functional activities of secreted cytokines.[12-16]

It could be stated that genetic variance in several populations can influence the innate, inflammatory, and immunological response to microbial infections and may contribute to genetic susceptibility to aggressive periodontitis. Since 1997, many studies in populations of different origins have evaluated the efficacy of SNPs of cytokines in periodontitis; some results indicated positive effect and others showed no relationship.[17,18]

In Iranian-Khorasani population with AgP, to the knowledge of the authors, this study was the first report of evaluating polymorphisms of IL-1β and TNF-α cytokines. Previously, we have analyzed IL-10, IL-1α and IL-1RN polymorphisms in association with AgP.[19-21] The present study sought to investigate the effect of IL-1β +3954 C/T and TNF-α -308 G/A SNPs on susceptibility to AgP.

MATERIALS AND METHODS

Study Population

In this study 118 unrelated, nonsmoking Iranian-Khorasani (North-East of Iran) subjects less than 35 years of age were selected by two periodontists in the clinic of periodontology of Mashhad Dental School. The diagnosis of generalized aggressive periodontitis (GAgP) was based on dental examination, medical and dental history, probing depth, assessment of attachment loss, tooth mobility and radiographs. Patients, demonstrated attachment loss of 5 mm or more in at least eight of their permanent teeth, three of which were not incisors or first molars. [21] No systemic disorders were present in neither periodontitis group nor periodontaly healthy group. The mean age of the GAgP group was 27.45 years (range: 18–35) and that of the control group was 29.75 years (range: 18–40). From 58 patients, 38 (65.5%) were female and 20 (34.5%) were male. From 60 control subjects, 37 (61.6%) were female and 23 (38.4%) were male. The laboratory procedure was conducted at Bu-Ali Research Institute. This study was approved by the ethical committee of the Mashhad University of Medical Sciences (MUMS) and written informed consent was obtained from participants.

Genotype Identification

Blood samples were taken by venipuncture from the arm vein of each subject and collected with EDTA as anticoagulant; genomic DNA was isolated using a "salting out" method with a commercial kit (BioGene, Mashhad-Iran). Genotyping was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique using specific primers and enzymes (Table 1). The amplification condition of each cytokine is described in Table 2. For PCR to occur, a CORBET thermocycler (Corbet Research, Australia) was used. The presence or absence of PCR products was visualized by ethidium bromide

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Table 1. Specific primers and restriction enzymes.

Cytokine SNP		Primers sequences	Enzyme	Cut sites
IL-1β	SENCE	5'-GTTGTCATCAGACTTTGACC-3'	*Taq 1*	T/CGA
+3954 C/T	ANTISENCE	5'-TTCAGTTCATATGGACCAGA-3'		
TNF-α -308	SENCE	5'-TCC TCC CTG CTC CGA TTC CG-3'	*Nco 1*	C/CATGG
G/A	ANTISENCE	5'-AGG CAA TAG GTT TTG AGG GCC AT-3'		G/GTACC

Table 2. PCR amplification conditions.

IL-1β	2cycles: 95°C, 2 min;68°C,1min;72°C,1min.
	35 cycles: 95°C, 2 min; 60°C, 1 min; 72°C ,1min.
	94°C , 1min; 68°C, 1min; 72°C, 5 min.
TNF-α	95°C, 2 min. 39 cycles; 95°C, 1 min; 62°C, 1 min; 72°C, 1 min.
	72°C, 5 min

using 1.5% agarose gel electrophoresis. PCR products of IL-1 and TNF then were digested by restriction enzymes *Taq 1* and *Nco 1* (Fermentas, Germany), respectively. Cleaved DNA fragments were subjected to electrophoresis in 17% polyacrylamide gel for IL-1β and in 2.5% agarose gel for TNF-α. The final products were stained with silver nitrate (IL-1β) and ethidium bromide (TNF-α).

Data Analysis

The frequency of alleles and genotypes were in Hardy-Weinberg equilibrium. To analyze the difference between genotype and allele frequencies in two groups, the chi-square (χ^2) test was used by SPSS (V.15) software. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Figures1 and 2demonstrate the homozygote and heterozygote results of the PCR-RFLPs

Genotypes and Allele Frequency

TNF-α -308

The frequency of GG, GA and AA genotypes in periodontitis group (n=58) were 24, 26 and 8, respectively and in control group (n=60) were 30, 28 and 2, respectively.

No statistically significant difference was found between the two groups (p=0.116). In periodontitis group and control group, the frequency of C and T alleles were 74, 42 and 88, 32, respectively. No statistically significant difference was found between the two groups (p=0.114, Table 3).

IL-1β +3954

The frequency of CC, CT and TT genotypes in periodontitis group (n=53) were 32, 21 and 0, respectively and in periodontaly healthy (control) group (n=48) were 24, 20 and 4, respectively. No statistically significant difference was found between two groups. In periodontitis group and normal group, the frequency of C and T alleles were 85, 68 and 21, 28, respectively. No statistically significant difference was found between the two groups (p = 0.121, Table 4).

In IL-1β assessment, some samples failed after several tests. Hence the numbers of cases and controls differ for the the two investigations.

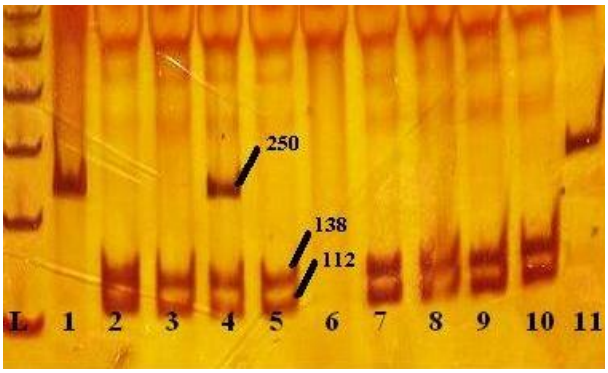

Figure 1. Results of restriction enzyme *Taq1* on IL-1β PCR product: Uncut TT: 1 & 11. Homozygote cut CC: 2, 3, 5, 7, 8, 9 & 10. Heterozygote cut CT: 4. 6: Failed. L: Ladder

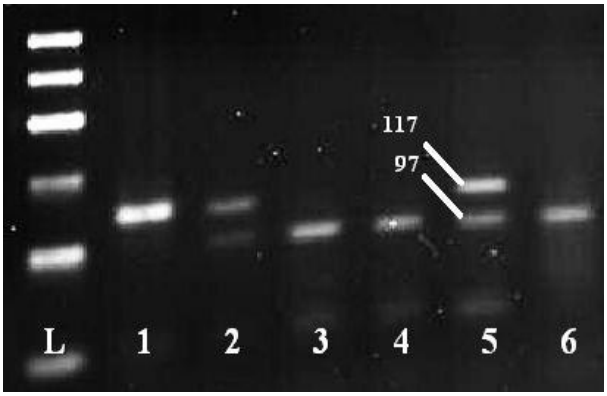

Figure 2. Results of restriction enzyme *Nco1* on TNF-α PCR product: Uncut AA: 1. Homozygote cut GG: 3, 4, & 6. Heterozygote cut GA: 2 & 5 L: ladder
The third band of heterozygote cut was not detectable due to low size (20bp).

Table 3. Frequency of TNF-α -308 genotypes and alleles in GAgP and controls.

	Periodontitis n (%)	Controls n (%)
GG	24 (41.4%)	30 (50%)
GA	26 (44.8%)	28 (46.7%)
AA	8 (13.8%)	2 (3.3%)
Total	58 (100%)	60 (100%)
Allele G	74 (63.8%)	88 (73.3%)
Allele A	42 (36.2%)	32 (26.4%)
Total	116 (100%)	120 (100%)

χ^2 test showed no significant differences in two groups for allele/ genotype frequency (p = 0.114).

Table 4. Frequency of IL1β +3954 genotypes and alleles in GAgP and controls.

	Periodontitis n (%)	Controls n (%)
CC	32 (60.4%)	24 (50%)
CT	21 (39.6%)	20 (41.7%)
TT	0 (0.0%)	4 (8.3%)
Total	53 (100%)	48 (100%)
Allele C	85 (80.2%)	68 (70.9%)
Allele T	21 (19.8%)	28 (29.1%)
Total	106 (100%)	96 (100%)

χ^2 test showed no significant differences in the two groups for allele/genotype frequency (p = 0.121).

DISCUSSION

It is a commonly held opinion that genetic variance is a major characteristic for complex diseases such as periodontitis. While microbial pathogens initiate periodontitis, individuals are known to respond differently to common environmental factors. In the last decade, numerous periodontal research projects were performed to investigate well-known genetic risk factors in the innate immune system. Cytokine gene polymorphisms have an effect upon immune response harmony, leading to different functional role, which in turn supposedly influence the outcome of periodontitis establishment and evolution.[22]

In this study we evaluated the functional gene polymorphisms of IL-1β (+ 3954 C/T) and TNF-α (-308 G/A) for their association with GAgP. Results showed no correlation between occurring polymorphisms and susceptibility to GAgP.

Consistent to our findings, many studies failed to prove the contribution of cytokine alleles to disease risk. Fiebig and colleagues, studied 9 polymorphisms of IL-1 gene cluster, including IL-1α -889, IL-1β +3954 and IL-1RN-VNTR, within 415 AgP patients and 874 controls in a Caucasian population.[23] Their results did not support the opinion that the tested variants in the IL1 gene were associated with AgP in smokers, non-smokers and controls. Sakellari and colleagues investigated the prevalence of 5 SNPs (IL-1α+4854, IL-1β+3954, TNF-α-308, IL-1RN–VNTR and col1A1SP1) alone, or as composite genotypes in ChP (n=56), AgP (n=46) and non-periodontitis (n=90) in a Greek population.[24] No statistical difference was observed at all comparisons. Gore and colleagues evaluated 3 SNPs (IL-1β +3953, IL-1β -511 and IL-1α -889) in early and moderate ChP (n=20), advanced ChP (n=12) and matched controls (n=32) in a Caucasian population.[25] The difference between combined genotype in all groups did not reach significance; likewise, when the genotypes were separately analyzed, neither IL-1α nor IL-1β -511 showed a significant association with the disease. Only IL1β +3953 homozygote genotype frequency showed significant decrease in advanced ChP compared to other groups, suggesting playing a role in severity of ChP. Kaarthikeyan and colleagues concluded the relationship between Chp (n=30) and healthy individuals (n=31) in India had insignificant difference.[26] Craandijk and colleagues studied 4 SNPs of TNF-α (-376, -308, -238

and +489) in ChP (n=90) and control group (n=264) in a Dutch population.[27] They concluded there was no indication that carriage of TNF-α A allele was associated with increased susceptibility to ChP. Menezes and Colombo appraised TNF- α -308 among ChP (n=74), GAgP (n=38) and healthy individuals (n=51). They negotiated the lack of association between periodontal disease and polymorphism of TNF-α in Brazil.[28] Guzeldemir and colleagues evaluated the gene polymorphisms of IL-1 and TNF-α in 31 localized AgP and 31 healthy persons. They also noted no relationship between TNF-α polymorphism and risk of periodontitis.[29]

Although our study and other numerous studies failed to prove the role of mentioned SNPs in periodontitis, a few studies have shown a positive relationship. Soga and Colleagues examined 5 SNPs for TNF-α (-1031 C/T, -863 C/A, -857 C/T, -308 G/A, -238 G/A) and 3 SNPs for IL-1β (-511 T/C, + 3953 C/T, -31 C/T) in 64 ChP and 64 healthy subjects in a Japanese population. They concluded that composite genotypes of TNF-α SNPs (-1031T, -863A and -857T) were significantly higher in severe ChP than healthy subjects.[30] McGuire and Nunn tested 42 periodontitis patients in an American population, and indicated that both IL-1 genotype positive (2.7 times) and heavy smoking (2.9 times) were significantly related to tooth loss. The combined effect of IL-1 positive genotype and heavy smoking increased the risk of tooth loss 7.7 times.[31] Erciyas and colleagues investigated 35 patients with GAgP in comparison to 85 healthy persons for impact of SNPs (IL-6, IL-10, IFN-gamma, TGF-ss1 and TNF-α) in Turkey. They pointed out that only TNF-α -308 A may be associated with development of AgP.[32]

Collectively, by reviewing the literatures, it becomes evident that the studies performed to evaluate the effect of SNPs on periodontitis have controversial results. These dissimilarities might be due to the different type of periodontitis, dissimilar sample sizes and genotype frequency variances in populations with different origins. By way of illustration, there is diversity in distribution of polymorphic alleles in "healthy" individuals of several populations explaining why the results of one study population could not be extrapolated to the others and why such studies are needed to explore the role of cytokine gene polymorphisms in pathogenesis of periodontitis via a number of inhabitants. The prevalence of IL-1β +3954 T allele in Iran (this study) is 29.1%, in Australia 40%,[31] in Turkey 88.7%,[29] in Brazil 17.6%,[33] in South Africa 20%, [34] in China 53.7%,[35] in India 20.9%,[36] in the Netherland is 33.8%[37] and in Italy is 21.6%.[38] The frequency of TNF-α -308 A allele in randomized populations of Iran, Turkey,[29] India,[39] Brazil,[33] Australia,[40] China,[41] Malaysia,[42] Mexico[43] and Ireland[44] are 26.4%, 11.3%, 90.6% , 27.5%, 14% , 26.6%, 4.9%, 20% and 23%, respectively. These differences could to some extent, explain why the polymorphisms in some populations are associated with AgP but not in others.

Furthermore, interactions with other important genes of components of immune response may play an important role. The frequencies of these genes are probably different among populations. As a result various degrees of disease-gene association could be observed among populations. Besides, environmental risk factors and habits such as smoking or oral hygiene habits may be quite different among populations. Such differences could add to the resulting variance in the pattern of expression of multifactorial diseases such as AgP.

Our results demonstrated that gene polymorphisms of IL-1β +3954 and TNF-α -308 may not significantly contribute to the GAgP incidence and brings into doubt the usefulness of these candidate gene SNPs as a screening tool for susceptibility to periodontitis in Iranian population. Moreover, further research based on all components of immune response and gene-environment interactions are needed to substantiate the allegations for genetic susceptibility of AgP.

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