

Association of Tumor Necrosis Factor Receptor Type 2 Gene Polymorphism with Severe Chronic Periodontitis

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Abstract

Background and aims. The aim of the present study was to evaluate the association between TNFR2 (+587T/G) gene polymorphism and chronic periodontitis (CP).

Materials and methods. One hundred and seventy-four non-smoking patients (35-72 years of age) with chronic periodontitis were selected according to established criteria and divided into three groups according to their probing pocket depth (PPD), clinical attachment level (CAL), and alveolar bone loss (ABL). Single nucleotide polymorphism at position +587 (T/G) in the TNFR2 gene was detected by a polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method.

Results. The distribution of genotypes for TNFR2 polymorphism at position +587 was not significantly different between moderate and severe chronic periodontitis patients compared with controls ($P=0.33$). In addition, the frequency of the +587 allele was not associated with the number of teeth ($P=0.58$), mean PPD ($P=0.9$), mean CAL ($P=0.94$), mean ABL ($P=0.99$) and bleeding on probing (BOP) ($P=0.07$) in the patients studied.

Conclusion. This study suggests that there is no correlation between genotype and severity of chronic periodontitis.

Key words: Bone loss, chronic periodontitis, cytokine, gene, polymorphism.

Introduction

Periodontitis is a chronic infectious disease of the supporting tissues of teeth. It affects 10-15% of the adult population and represents the major cause of tooth loss in adults.¹

The study of genetic markers associated with the severity and susceptibility of periodontal disease has recently received considerable attention. Polymorphisms in genes encoding molecules of the host defense system, such as cytokines, have been suggested as potential genetic markers.²⁻⁴

Proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) have been found at high levels in gingival crevicular fluid and gingival tissues from periodontitis-affected sites in humans, inducing tissue destruction and bone resorption.⁵⁻⁶

TNF- α has diverse biologic effects by binding two high-affinity cell surface receptors, TNF receptors type 1 and type 2 (TNFR1, TNFR2). TNFR1 mediates the TNF signal to cells and TNFR2 enhances this activity by binding to TNF, then passing it onto TNFR1 (ligand passing). Therefore, TNFR2 would increase the sensitivity of a cell to TNF stimulation.

The extracellular domain of TNFR2 results in the generation of soluble forms of TNFR2. This soluble TNFR2 can compete for TNF with the cell surface receptors and block the cytokine activity. Therefore, the role of TNFR2 and soluble TNFR2 is not limited to signal transduction but includes extracellular regulatory functions affecting TNF bioavailability.⁷

Recent studies have also shown allelic variation in cytokine genes and factors influencing the clinical outcome, susceptibility and progression of periodontal disease.^{2,4,8,9}

Current data on cytokine gene single nucleotide polymorphisms (SNPS) in periodontitis is incomplete and sometimes inconsistent because studies have been performed on patients with different racial and ethnic backgrounds and further studies are still necessary to determine clear associations. Therefore, in this study, distributions of TNFR2 gene polymorphism in Iranian subjects with different levels of chronic periodontitis were investigated.

Materials and Methods

The study population consisted of 174 untreated subjects (35-72 years of age) with different levels of chronic periodontitis in the Department of Periodontics, Shiraz Faculty of Dentistry. The participants signed consent forms before entering the study.

The exclusion criteria consisted of the following: history of or current manifestations of systemic dis-

ease, diabetes, hepatitis, pregnancy lactation, autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), current use of antibiotics during the past 3 months and smoking.

All the patients were evaluated clinically and radiographically during the first visit to assess the following periodontal measurements: number of teeth, probing pocket depth (PPD), clinical attachment level (CAL), supragingival plaque accumulation with Loe and Sillness index, bleeding on probing (BOP) and with simplified index and alveolar bone loss (BL).

The subjects were classified into the following:

- (1) Severe chronic periodontitis (CP): subjects having more than seven interproximal sites with $\geq 50\%$ BL and total mean BL of $>34\%$.
- (2) Moderate CP: subjects having less than three interproximal sites with $\geq 50\%$ BL and total mean BL of 16-34%.
- (3) Controls: subjects having no PPDs >3 mm and no sites with BL $>15\%$.¹⁰

Clinical characteristics of the subjects are summarized in Table 1.

Blood Samples and DNA Extraction

Peripheral venous blood was collected in sterile tubes containing EDTA solution. Genomic DNA was isolated according to Miller's salting-out procedure with Wako pure kit.

Genotype of TNFR2 gene polymorphism in 35-70 ng of DNA sample was amplified in 25 μ L of the reaction mixture containing $\times 10$ reaction buffer: 1.5 mM f MgCl₂, 0.2 mM of dNTP, a pair of 0.75 μ M of each primer and 1.25 Utaq f polymerase. The polymerase chain reaction (PCR) products were analyzed by a PCR.

Restriction Fragment Length Polymorphisms (RFLP) Method

The primer sequences were as follows: sense 5'-ACT CTC CTA TCC TGC CTG CT-3'; antisense 5'-TTC TGG AGT TGG CTG CGT GT-3'.

Table 1. Clinical features of chronic periodontitis

Feature	Mild CP (control)	Moderate CP	Severe CP
Age	42.4 \pm 7.3	45.1 \pm 7.8	46.6 \pm 9.2
Male/female	12/58	24/51	13/29
Number of teeth	26.4 \pm 4.1	26.6 \pm 3.3	22.4 \pm 6.4
PPD (mm)	2.52 \pm 0.7	3.20 \pm 0.7	4.09 \pm 0.9
CAL (mm)	2.80 \pm 0.7	3.63 \pm 0.8	5.07 \pm 1.0
BL (%)	11.29 \pm 3.9	25.13 \pm 4.4	43.55 \pm 7.9
Site with plaque (%)	65.3 \pm 26.4	79.3 \pm 23.5	86.02 \pm 20.0
Site with BOP (%)	72.5 \pm 32.0	74.9 \pm 22.7	81.1 \pm 28.5

All values except for male/female ratio are expressed as mean \pm SD (standard deviation).

CP: Chronic periodontitis; PPD: probing pocket depth; CAL: clinical attachment level; BL: bone loss; BOP: bleeding on probing.

The PCR conditions were: Initial denaturation for 10 min at 95°C, followed by 35 cycles of 1-min denaturing at 95°C, 1-min annealing at 60°C, 1-min extension at 72°C, and a final extension cycle at 72°C for 5 min. The PCR products were checked by 2% agarose gel electrophoresis and then, digested with 5U of HSP92II at 37°C overnight. The restriction fragments were determined on 3% agarose gel electrophoresis, stained with ethidium bromide.

Statistical Analysis

Allelic and genotypic frequencies were obtained by direct counting. The association of allele and genotype frequencies was analyzed by chi-squared test. The association of gender with genotypes polymorphism and number of teeth with genotypes and late clinical parameters such as CAL, BL, PI and BI with genotypes were determined by sequential Mann-Whitney *U*, Kruskal-Wallis and Spearman tests. The odds ratio (OR) was calculated along with its 95% CI. Statistical significance was defined at $P < 0.05$.

Results

Allelic and genotypic frequencies were obtained by direct counting. Distribution of TNRF2 genotypes in Iranian non-smoking subjects with different levels of chronic periodontitis were evaluated as shown in Table 1. The genotype and allele frequencies are shown in Tables 2 and 3.

No statistically significant differences were detected in genotype between the three groups ($P=0.7$) and moderate and severe chronic periodontitis ($P=0.12$). In addition, the allelic distribution did not exhibit statistically significant differences between mild chronic periodontitis and the two other groups ($P=0.4$). No statistically significant differences were found between genotype distribution and number of remaining teeth ($P=0.58$), probing depth ($P=0.9$), clinical attachment level ($P=0.94$), bone loss ($P=0.99$), plaque index ($P=0.4$) and bleeding index ($P=0.07$).

Discussion

There are several reports of increased levels of cytokines in the gingival crevicular fluid and gingival tis-

Table 3. Allelic distribution in chronic periodontitis (CP) patients

Allele frequency	Control		Moderate CP		Severe CP		P value
	%	N	%	N	%	N	
G	31.0	41	25	33	38.0	32	0.14
T	68.9	91	75	99	61.9	52	

Statistical significance at $P < 0.05$.

sues of periodontitis patients. In particular, polymorphisms in genes encoding these cytokines have been targeted as potential genetic markers. In this study, no association was found between TNFR2 gene polymorphism and severity of chronic periodontitis in non-smoking Iranian patients ($P=0.14$).

However, in a study carried out by Shimada on non-smoking Japanese patients the frequency of the allele G at +587 gene was greater in severe chronic periodontitis patients compared with mild chronic periodontitis patients ($P=0.006$).¹⁰ The frequency of the allele G at this gene area in healthy subjects was 45% in Caucasians,¹¹ 33% in African-Americans,¹² 17% in Japanese,¹⁰ and 31% in Iranian subjects. The amount of this allele at +578 gene in our healthy subjects was very similar to African-Americans, lower than Caucasians but greater than the Japanese data.

In multiple risk factor syndromes such as periodontitis, a variety of genes might influence the individual's susceptibility or severity of the disease. Since TNF- α has been suggested to participate in the establishment of inflammatory lesions in periodontitis, TNF- α might be one of the important genes associated with the severity of periodontitis. SNP of TNF- α , -1031,-836, -857 have been suggested to be related to high TNF- α production and severity of periodontitis.¹³ Although reports on the genetic polymorphisms associated with periodontal disease are increasing one can summarize that there is serious inconsistency in particular polymorphisms in different studies. Apart from racial differences, this might be attributed to many confounding factors, including clinical diagnosis, environmental variables, biologic plausibility, and logic of associated studies.

The observations made in the present study demonstrated no association of TNFR2 genotype with periodontitis in this Iranian population.

Further studies are warranted with larger sample

Table 2. Distribution of the TNFR2 gene polymorphism in chronic periodontitis (CP) patients

Genotype	Mild CP (control)		Moderate CP		Severe CP		P value
	%	N	%	N	%	N	
GG	13.6	9	6.06	4	16.6	7	0.33
GT	34.8	23	37.8	25	42.8	18	
TT	51.5	34	56.0	37	40.4	17	

Statistical significance at $P < 0.05$.

sizes to further evaluate and the link between cytokine polymorphisms and periodontitis.

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