Original Article

Interleukin (IL)-17F (H161R) and IL-23R (R381Q) Gene Polymorphisms in Turkish Population with Periodontitis

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ABSTRACT

Background: Periodontitis is triggered by periodontal pathogens and influenced by environmental and genetic factors. Genes encoding molecules related to the immune response are the main candidates for polymorphisms analysis and may be possibly associated with this pathology.

Aim: The aim of the study was to evaluate the interleukin *(IL)-17F* Histidine161Arginine (H161R) and *IL-23R* Arginine381Glycine (R381Q) gene polymorphisms in patients with periodontitis in Turkish population.

Materials and Methods: 90 periodontally healthy, 90 patients with chronic periodontitis and 57 patients with generalized aggressive periodontitis were included in the study. Participants were identified through clinical examinations and radiographs. DNA was isolated from venous blood samples from each patient and genotype analyses were made for single nucleotide polymorphisms (SNPs). Data were analyzed using the x^2 test.

Results: The comparison of allelic, genotypic frequencies of the *IL-17F* (H161R) and *IL-23R* (R381Q) polymorphisms revealed no significant differences between the periodontally healthy individuals and patients with periodontal diseases.

Conclusion: On the basis of the present findings, it can be suggested that *IL-17F* gene (H161R) and *IL-23R* gene (R381Q) polymorphisms are not associated with the susceptibility to periodontitis in Turkish population.

Key Words: Periodontitis, cytokines, polymorphism, interleukin-17F, interleukin-23R

INTRODUCTION

Periodontitis is an inflammatory disease that affects the integrity of tooth-supporting tissues and one of the major causes of tooth loss. Although periodontal diseases are initiated by bacteria, the host response plays a crucial role in the breakdown of the connective tissue and bone. The host response is influenced by several possible risk factors such as oral hygiene, smoking, age, gender, and genetic factors [1]. The genetic background may affect many functions including innate and adaptive immunity, bacterial colonization and other modifying factors. Genetic researches in periodontal disease have focused on single nucleotide polymorphisms (SNPs) of genes encoding cytokines, cell-surface receptors, chemokines or enzymes [2]. The appearance or the severity of periodontitis may be affected by the genetic control of the cytokine function. Several studies have investigated gene polymorphisms of the cytokines in chronic or aggressive periodontitis [3-5]. Allelic variations on cytokine genes and factors affecting their releasing have caused phenotypic differences in cytokine response among individuals and this is crucial on progression of disease and sensitivity to disease [6].

Interleukin (IL)-17 is a novel cytokine family with a proinflammatory nature, and consists of similar cytokine members designed from IL-17A-F, according to the order in which they were discovered [7]. These cytokines have been associated with the pathogenesis of an extensive list of inflammatory and autoimmune diseases including psoriasis, rheumatoid arthritis, inflammatory bowel disease [8,9] and periodontitis [10,11]. IL-17 plays a role in activation of T cells, fibroblasts and osteoclast, dendritic cells maturation. IL-17 induces the expression of proinflammatory cytokines, chemokines and metalloproteinases to coordinate tissue inflammation [12].

IL-23 is a recently discovered cytokine belonging to the IL-12 family and one of the upstream regulators

of IL17-family members [13]. This cytokine stimulates the production of IL-1β and tumor necrosis factor (TNF)-α in host defense against pathogens; upregulates the expression of matrix metalloproteinase (MMP)-9; is effective on activation, survival and expansion of Th17 cells by inducing the secretion of IL-17 family cytokines and enhances the proliferation of memory T cells [14]. The level of IL-23 was correlated with periodontitis disease activity and elevated in periodontitis-affected tissues [10].

Association analysis of SNP in *IL-23R*, *IL-17F* and *IL-17A* has been performed in several inflammatory diseases [15-17]. In vitro functional analysis revealed that the His-to-Arg substitution at amino acid 161 (H161R) in the 3rd exon (rs763780, 7488T/C) of the *IL-17F* gene causes loss of the ability of IL-17F to induce expression of certain cytokines and chemokines [17] and was found to be associated with asthma and inflammatory bowel disease [18,19]. Several polymorphisms have been described in IL-23R gene, however, only the R381Q (rs11209026, 1142G/A) polymorphism, whose minor allele plays a protective role for several autoimmune diseases, appear to have a functional involvement [16]. To the best of our knowledge, this is the first study evaluating *IL-17F* and *IL-23R* gene polymorphisms in patients with periodontitis in Turkish population. We aimed to determine the allelic and genotypic frequencies of *IL-17F* gene (H161R) and *IL-23R* gene (R381Q) polymorphisms in Turkish population with periodontitis.

MATERIALS AND METHODS

All participants were recruited from the Department of Periodontology, Faculty of Dentistry, Kirikkale University from July 2009 to June 2012. Before participation, all participants were informed about study goal and procedures and all participants gave written informed consent in accordance with Helsinki Declaration. The study protocol was approved by the Ethics Committee of the Kirikkale University. Patients with systemic diseases such as diabetes mellitus, immunologic or blood disorders, hepatitis, pregnant and lactating women, smokers were excluded. None of the participants received antibiotics or anti-inflammatory drugs regularly within the previous 3 months or treatment for periodontal disease within the last 6 months prior to the study. The dentition of each volunteer was examined clinically and radio- graphically to assess the suitability of the participants for the study. The selected patients were of the same ethnic origin.

Study Groups

Study population was classified into three groups based on their periodontal conditions according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions [20].

1. Generalized Aggressive Periodontitis (GAgP): The generalized aggressive periodontitis group included 57 patients (25 females and 32 males) (mean age:39.6±1.8 years) who had demonstrated a generalized pattern of severe destruction and clinical attachment loss (CAL) of ≥5 mm and probing depth (PD) ≥6mm on 8 or more teeth; at least 3 of those were other than central incisors or first molars.
2. Chronic periodontitis (CP): The chronic periodontitis group included 90 patients (49 females and 41 males) (mean age: 47.3±2.3 years) who had moderate to severe alveolar bone loss and CAL of ≥ 5 mm and PD of ≥ 6 mm in multiple sites of all four quadrants of the mouth, but with no evidence of rapid progression.

Periodontally healthy (H): A total of 90 systemically and periodontally healthy participants (43 females and 47 males) (mean age: 34.7±1.2 years) were included in the healthy control group. Healthy participants had no sites with PD >3 mm and CAL, a BOP score of <15% at the examination, and no alveolar bone loss was observed.

Clinical periodontal parameters

PD and CAL were determined at six sites of per tooth; plaque index (PI) [21] and gingival index (GI) [22] were determined at four sites of per tooth in the whole mouth, excluding third molars. A manual William's periodontal probe (Hu-Friedy, Chicago, IL) was used for PD and CAL measurements. All measurements were performed by a calibrated examiner (MKH). Genotyping for *IL-17* gene H161A and *IL-23R* gene R381Q Polymorphisms. Three milliliters of peripheral venous blood samples were collected via a single venipuncture and stored at -20 ^{0}C until genetic analyses were performed. Genomic DNA was extracted from peripheral leukocytes using the Bio Basic DNA Isolation Kit (Bio Basic Inc, Ontario, Canada) and used as a template for genotyping procedures. Genotyping for *IL-17* gene H161A and *IL-23R* gene R381Q polymorphisms were performed by real-time PCR mutation detection/allelic discrimination kit (PrimerDesign® Ltd, UK) following the manufacturer's instructions. Briefly, ~ 20 µl PCR reactions were carried out containing 1–10 ng DNA template, 1 µl genotyping primer/probe mix, 10 µl 2x PCR Master Mix. The reaction mixture was amplified according to the

recommendation of the supplier. The wild-type probe is labeled to read through the ROX channel whilst the mutant probe is labeled to read through the VIC channel. The genotype of each sample is calculated by comparing the ratio of signals between the two channels (ROX and VIC).

Statistical Analyses

Non-parametric techniques were used for statistical analysis and Kruskal-Wallis test was performed for comparisons among the study groups. When significant differences were observed ($p<0.05$), post hoc two-group comparisons were identified with Bonferroni-corrected Mann-Whitney U tests, and $p<0.05$ was considered to be statistically significant. Genotype distributions and allele frequencies were analyzed by χ^2 test. All data analyses were performed using a statistical package (SPSS for Windows Ver. 15.0, Chicago, IL). Statistical significance level $p<0.05$ was significant considered.

RESULTS

The intra-examiner reliability was high as revealed by an intra-class correlation coefficient of 0.82 and 0.80 for PD and CAL measurements, respectively. The demographic characteristics of the study groups were presented in Table 1.

Table 1: Demographics characteristics of the study groups

Demographic Characteristic	CP	GAgP	H
Age (years; mean±SE)	47.3±2.3*	39.6±1.8	34.7±1. 2
Sex Female	49	25	43
Male	41	32	47

*Significant difference from healthy group ($p<0.05$)

All clinical periodontal parameters were significantly higher in patients with CP and GAgP than those of the H group. The AgP group had significantly higher mean PD and CAL scores compared to the CP group ($p<0.05$).

The *IL-17F* genotypes of the CP group (TT 90.4%; TC 8.4% and CC 1.2%) and GAgP (TT 93.0%; TC 7.0% and CC 0%) were not significantly different from the frequencies observed in the H group (TT 93.3; TC 5.6% and CC 1.1%) ($p>0.05$). T and C allele frequencies for chronic periodontitis were 0.95 and 0.05; 0.96 and 0.04 for aggressive periodontitis, respectively. However, these frequencies were not significantly different when compared to periodontally healthy group (Table 2).

Table 2: Genotype and allele frequencies of IL-17F(H161R) and IL-23R(R381Q) polymorphisms in study groups

	Genotype n (%)			p	Allele (%)	
IL-17F (H161R)	TT	TC	CC		T	C
CP	75 (90.4)	7 (8.4)	1 (1.2)	0.87	0,95	0,05
GAgP	53 (93.0)	4 (7.0)	0(0)	0.79	0,96	0,04
H	83 (93.3)	5 (5.6)	1 (1.1)	0.53	0,96	0,04
IL-23R (R381Q)	GG	GA	AA		G	A
CP	75 (90.4)	7 (8.4)	1 (1.2)	0.44	0,95	0,05
GAgP	48 (92.3)	4 (7.7)	0 (0)	0.35	0,96	0,04
H	85 (95.5)	3 (3.4)	1 (1.1)	0.10	0,98	0,02

$p \leq 0.05$ (x^2 test)

The *IL-23R* genotypes of the CP group (GG 90.4%; GA 8.4% and AA 1.2%) and GAgP (GG 92.3%; GA 7.7% and AA 0%) were not significantly different from the frequencies observed in the H group (GG 95.5; GA 3.4% and AA 1.1%) ($p>0.05$). G and A allele frequencies for chronic periodontitis were 0.95 and 0.05; 0.96 and 0.04 for aggressive periodontitis, respectively. However, these frequencies were not significantly different (Table 2). The distribution of genotype frequencies were in Hardy-Weinberg equilibrium.

DISCUSSION

In scientific literature, the enhancement in the number of reports related to SNP in various inflammatory diseases is noteworthy. In periodontal diseases, the determination of allelic variants of genes may be used to assess the risk of disease. Many researchers have estimated that polymorphism information would be helpful in prevention and treatment of periodontitis. Cytokine gene polymorphisms may create phenotypic differences in the cytokine response among individuals and that is important for individual's susceptibility to disease, progression of disease or response to treatment. This is the first scientific study evaluating *IL-17F* gene (H161R) and *IL-23R* gene (R381Q) polymorphisms in patients with chronic and aggressive periodontitis in Turkish population. There were no significant differences of allelic frequencies for both polymorphisms among groups.

IL-17 has pleiotropic biological activities on various cell types, such as fibroblasts, endothelial cells and epithelial cells [23] and a stimulator effect on osteoclastic bone resorption [24]. It was revealed

that IL-17 was participated in the development of gingival inflammatory response by arranging the activation of immune response in patients with periodontitis [11]. IL-17F has a regulatory role in immune response initiated by T-cells and it is a member of IL-17 cytokine family [25]. In a study evaluating the effect of *IL-17F* His161Arg polymorphism in inflammatory bowel disease, it was shown that the genotype frequencies were similar between patients and healthy individuals and this polymorphism did not affect the phenotypes of the disease [26]. Similarly, in our study, genotype and allele frequencies of *IL-17F* His161Arg polymorphism were similar in both periodontitis and periodontally healthy individuals. In contrast, in the study analyzing the relationship between severity of the disease and genotype frequencies at different points for *HLA-DRB1*, *RANK*, *RANKL*, *OPG*, and *IL-17*, it was demonstrated that *RANKL* (rs2277438) and *IL-17* (rs3804513) SNPs were associated with the radiographic progression of disease in Japanese patients with rheumatoid arthritis [27].

IL-23 is secreted by activated dendritic cells, monocytes, and macrophages, and IL-23R is a potent proinflammatory cytokine and a key regulator of Th17 cells, which plays a central role in mediating chronic inflammatory responses [28]. In a study evaluating the expression of IL-17A and occurrence of the *IL-17A* (rs2275913), *IL-17F* (rs763780) and *IL23R* (rs11209026) gene polymorphisms in different clinical forms or severity of periodontitis in a sample of Brazilian individuals, *IL-17A* expression was higher in control group and *IL-17A* (rs2275913) gene polymorphism was found to be associated with aggressive and chronic periodontitis, the GG genotype and G allele were more frequent in the disease groups than controls. With regards to the *IL-17F* (rs763780) polymorphism there was no statistically significant difference among groups, in agreement with our study. Concerning *IL-23R* (rs11209026), a low frequency of AA genotype was observed in the disease group but no association was found with the clinical forms evaluated [29]. However, *IL-23R* (rs11209026) plays a protective role in IL-23-mediated IL-17A-induced peripheral tissue pathology in chronic inflammatory disease [16]. In our study, it was indicated that *IL-23R* (R381Q) polymorphism was not associated with the disease in individuals with chronic and aggressive periodontitis.

CONCLUSION

As a result, even though cytokine gene polymorphisms may cause phenotypic differences in cytokine response among individuals and affects individual's sensitivity to disease, progression of disease and response to the treatment; our results show that *IL-17F* gene (H161R) and *IL-23R* gene (R381Q) polymorphisms were not associated with periodontal disease in Turkish population. Further studies will be necessary to understand the functional role of *IL-17F* and *IL-23R* gene polymorphisms in periodontitis as well as other gene sequences of related cytokines.

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REFERENCES

1. Dentino A, Lee S, Mailhot J, Hefti AF. Principles of periodontology. Periodontol 2000 2013;61:16-53.
2. Kinane DF, Hart TC. Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med 2003;14:430-49.
3. Chambrone L, Ascarza A, Guerrero ME, Pannuti C, de la Rosa M, Salinas-Prieto E et al. Association of-1082 interleukin 10 gene polymorphism in Peruvian adults with chronic periodontitis. Med Oral Patol Oral Cir Bucal 2014;19:569–73.
4. Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. J Clin Periodontol 2008;35:754-67.
5. Ebadian AR, Radvar M, Tavakkol Afshari J, Sargolzaee N, Brook A, Ganjali R, et al. Gene polymorphisms of TNF-α and IL-1β are not associated with generalized aggressive periodontitis in an Iranian subpopulation. Iran J Allergy Asthma Immunol 2013;12:345-51.
6. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999;1:3-19.
7. Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol 2004;114:1265-73.
8. Abraham C, Cho J. Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis 2009;15:1090-100.
9. Kirkham BW, Kavanaugh A, Reich K. Interleukin-17A: a unique pathway in immune-mediated diseases: psoriasis, psoriatic arthritis and rheumatoid arthritis. Immunol 2014;141:133-42.
10. Lester SR, Bain JL, Johnson RB, Serio FG. Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss. J Periodontol 2007;78:1545-50.

11. Takahashi K, Azuma T, Motohira H, Kinane DF, Kitetsu S. The potential role of interleukin-17 in the immunopathology of periodontal disease. J Clin Periodontol 2005;32:369-74.
12. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 2010;10:479-89.
13. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity 2004 ;21:467-76.
14. Honda T, Aoki Y, Takahashi N, Maekawa T, Nakajima T, Ito H, et al. Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. Clin Chim Acta 2008;395:137-41.
15. Arisawa T, Tahara T, Shibata T, Nagasaka M, Nakamura M, Kamiya Y, et al. The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. J Clin Immunol 2008;28:44-9.
16. Di Meglio P, Di Cesare A, Laggner U, Chu CC, Napolitano L, Villanova F, et al. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effectors response in humans. PLoS One 2011;6:17160.
17. Kawaguchi M, Takahashi D, Hizawa N, Suzuki S, Matsukura S, Kokubu F, et al. IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. J Allergy Clin Immunol 2006;117:795-801.
18. Zhang X, Yu P, Wang Y, Jiang W, Shen F, Wang Y, et al. Genetic polymorphisms of interleukin 17A and interleukin 17F and their association with inflammatory bowel disease in a Chinese Han population. Inflamm Res 2013;62:743-50.
19. Maalmi H, Beraies A, Charad R, Ammar J, Hamzaoui K, Hamzaoui A. IL-17A and IL-17F genes variants and susceptibility to childhood asthma in Tunisia. J Asthma 2014;51:348-54.
20. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
21. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
22. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
23. Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, et al. Human IL-17: a novel cytokine derived from T cells. J Immunol 1995;155:5483-6.
24. Van Bezooijen RL, Farih-Sips HC, Papapoulos SE, Löwik CW. Interleukin-17: A new bone acting cytokine in vitro. J Bone Miner Res 1999;14:1513-21.
25. Rutitzky LI, Lopes da Rosa JR, Stadecker MJ. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. J Immunol 2005;175:3920-6.
26. Seiderer J, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack C, et al. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. Inflamm Bowel Dis 2008;14:437-45.
27. Furuya T, Hakoda M, Ichikawa N, Higami K, Nanke Y, Yago T, et al. Associations between HLA-DRB1, RANK, RANKL, OPG, and IL-17 genotypes and disease severity phenotypes in Japanese patients with early rheumatoid arthritis. Clin Rheumatol 2007;26:2137-41.
28. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 2003;198:1951-7.
29. Saraiva AM, Alves e Silva MR, Correia Silva Jde F, da Costa JE, Gollob KJ, Dutra WO, et al. Evaluation of IL17A expression and of IL17A, IL17F and IL23R gene polymorphisms in Brazilian individuals with periodontitis. Hum Immunol 2013;74:207-14.

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