

Interleukin 17 Receptor Gene Polymorphism in Periimplantitis and Chronic Periodontitis

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Abstract- Gene polymorphism of cytokines influencing their function has been known as a contributing factor in the pathogenesis of inflammatory diseases of the tooth and implant supporting tissues. The aim of this study was to investigate the association of IL-17R gene polymorphism (rs879576) with chronic periodontitis and periimplantitis in an Iranian population. 73 patients with chronic periodontitis, 37 patients with periimplantitis and 83 periodontally healthy patients were enrolled in this study. 5cc blood was obtained from each subject's arm vein and transferred to tubes containing EDTA. Genomic DNA was extracted using Miller's Salting Out technique. The DNA was transferred into 96 division plates, transported to Kbioscience Institute in United Kingdom and analyzed using the Kbioscience Competitive Allele Specific PCR (KASP) technique. Chi-square and Kruskal Wallis tests were used to analyze differences in the expression of genotypes and frequency of alleles in disease and control groups (P -Value less than 0.05 was considered statistically significant). There were no significant differences between periodontitis, periimplantitis with AA, GG, GA genotype of IL-17R gene ($P=0.8239$). Also comparison of frequency of alleles in SNP rs879576 of IL-17R gene between the chronic periodontitis group and periimplantitis group did not revealed statistically significant differences ($P=0.8239$). The enigma of IL-17 and its polymorphism-role in periodontitis and periimplantitis is yet to be investigated more carefully throughout further research but this article demonstrates that polymorphism of IL-17R plays no significant role in incidence of chronic periodontitis and Periimplantitis.

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Introduction

Nowadays dental implants are becoming more used to carry out oral rehabilitations and replacement of missing teeth. However, in spite of high success rate, it is not without complications (1,2).

The most common complications of implants are: periimplant mucositis and periimplantitis. In both of these complications presence of inflammation is the main sign, but in periimplantitis we had a loss of bone support in addition to inflammation while periimplant mucositis had no sign of loss of bone support (3,4).

Microorganisms that cause periimplantitis is so similar to microbacteria that found in periodontal disease, such as the species of the red and orange complexes (4).

Other major etiological factors of failure of dental

implants are: genetic variations, occlusal overload and impaired healing. In addition to these factors, poor surgical technique, poor bone quality and poor prosthesis design can cause failure of implants (5,6).

It was investigated that mucositis appears in 80% of the patients and in 50% of the implants, while periimplantitis is observed in 28-56% of the patients, and in 12-43% of the implants (7). On the other hand incidence of periodontal disease can be related to the individual risk factors of each patient such as smoking, stress, diabetes, osteoporosis or genetic. Progression of periodontal disease depends on interaction of this risk factors and host immune system. Studies shows genetic is the most important factor impact on differences in the progression of periodontal disease in twins (8). Genes play an indispensable role in the severity of periodontitis

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by affecting the immune response (9). Monocytes and macrophages releasing cytokines (proinflammatory mediators) such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α) to protect against bacteria (3).

The cytokines are multifunctional proteins and glycoproteins that categorized as signaling molecules. They are produced by immunocompetent cells such as T lymphocytes and monocytes in local inflammatory tissue (10).

Interleukin-17 (IL-17) is a proinflammatory cytokine expressed by activated T cells, and its cDNA contains a 293-amino acid extracellular domain, 7 potential N-linked glycosylation sites, 7 potential N-linked glycosylation sites, a 21-amino acid carboxyl-proximal transmembrane domain, and a 525-amino acid cytoplasmic tail (11).

IL-17 induces nuclear factor kappa-B ligand (RANKL), and expression of intercellular adhesion molecule-1 (ICAM1), granulocyte macrophage colony-stimulating factor (GM-CSF), and prostaglandin E2 (12). It has been tested the hypotheses that IL-17 is produced in periodontal lesion and can increase inflammatory reaction via gingival fibroblast derived mediators in periodontal disease (13). It was also demonstrated that *Porphyromonas gingivalis* outer membrane protein (OMP) induced significant increased in production of IL-17 in periodontitis patients, and that after the stimulation; IL-17 was detected more in patients with periodontitis than in those with gingivitis (12). IL-17R is a receptor for IL-17. The IL-17R gene is located in chromosome 22q11. Eight different IL-17R transcripts are identified by northern blot analysis. IL-17 binds weakly to IL-17R, given the low concentration of IL-17 needed to elicit a biologic response. Inhibition assays revealed that antibodies to IL-17R blocked IL-17 biologic activity, such as the induction of IL-6 production by fibroblasts. (11) Researches indicated, that in periodontal disease, increased serum IL-6 was related with carriage of allele 2 for IL-1A, TNF- α .

To date no study has investigated the relationship between IL-17R polymorphism and occurrence of periimplantitis. The present article was conducted to fill this gap by analyzing rs879576 SNP of IL-17 receptor.

Materials and Methods

In this cross sectional study a total of 193 non-smoker individuals were recruited; 73 patients with chronic periodontitis, 37 patients with periimplantitis and 83 healthy individuals.

The subjects were collected from patients who had attended the Periodontology department at Shahid Beheshti University of Medical Sciences (Evin, Tehran, Iran). All individuals had given informed consent to involve in the study. All subjects were above 30 years old. All patients were examined with reproducibility of 95%.

Exclusion criteria were:

- Diseases other than periodontitis, periimplantitis and dental caries.
- Existing orthodontics therapy.
- General health problems such as: diabetes mellitus, hepatitis, HIV infection, chemotherapy.
- Pregnancy and lactation.
- Do not receive any antibiotics and anti-inflammatory drugs in past three month.
- Non-Iranian races.

In order to be included in chronic periodontitis group all subjects:

- Had to have at least 5 teeth in each quadrant (except third molars).
- Presence of clinical attachment level more than 3mm.
- Pocket probing depth was more than 4mm.
- Bleeding on probing of at least three teeth in at least two quadrants.

The control group (healthy individuals) consists of subjects:

- Had no history and clinical signs of periodontitis.
- Had probing pocket depths less than 3mm around any implants/teeth presented in their mouths.
- Had no radiographic signs of bone resorption.

The inclusion criteria for the periimplantitis group:

- No history of periodontitis.
- Presence of one or more implants with a minimum of 12 months loading period (14).
- Probing pocket depths more than 5mm (15).
- Presence of bleeding on probing (with/without suppuration) (15).
- Radiographic sign of crestal bone loss in at least one area around an implant (15).
- Exposure of at least two threads of the implant (15).

According to the ISI (Implant Success Index) classification, groups VI, VII and VIII were included in this study (16).

After classifying the three groups according to the mentioned criteria, 5cc blood was obtained from each subject's arm vein and transferred into falcon tubes containing EDTA. In order to conduct the laboratory stages blindly, all the tubes was assigned by a unique code, which specialized for each subject. DNA was extracted from fresh blood using Miller's Salting Out

technique according to the extraction kit's manufacturer instructions (CinnaGen Inc. Iran). The concentration of extracted DNA samples was analyzed by spectrophotometers (75ngr). The samples were transferred into 96 division plates and transported to KBioscience Institute in United Kingdom for genotyping the polymorphism using Competitive Allele Specific PCR (KASP) technique. The various chemical stages of this technique can be briefly observed and studied by visiting the institute's website at: www.kbioscience.co.uk/reagents/KASP.html.

Here is the sequence of mentioned allele on NCBI:

TGGTCGGCTGAGTAGATGATCCAGAC[C/T]TT
CCTGGGCTTCAGCGGTGGGGGA

While KBioscience used the opposite strand of DNA for genotyping and has analyzed [G/A] substitution in this position.

Statistical analysis was accomplished by SPSS version 19 software. Chi-square and Kruskal Wallis tests were used to analyze differences in the expression of genotypes and frequency of alleles in disease and control groups. (P-Value less than 0.05 was considered statistically significant.)

Results

Kbioscience Institute -located in U.K. assessed the polymorphism of IL-17R. There we had 193 blood samples including 83 taken from healthy individuals (known as Control group) including 43 females and 40

males with an age range 30 to 55 and a mean age was 38.4. Mean probing pocket depth of healthy group was 5.77 ± 0.81 and the mean attachment bone loss was 0.8 ± 0.14 ; 73 taken from the Chronic Periodontitis diagnosed group (known as CP group) containing 38 males and 35 females with an age range 30 to 69 and their mean age is 48.3. The mean probing pocket depth of chronic periodontitis group was 5.77 ± 0.81 and the mean attachment bone loss was 5.37 ± 0.73 ; and 37 samples taken from Periimplantitis diagnosed group (known as PI group) with age range between 30 to 60 with mean age 50.2 including 18 females and 19 males. Mean probing pocket depth of periimplantitis group was 6.90 ± 0.35 and the mean attachment bone loss was 4.62 ± 1.84 .

In tables 1 and 2 comparing CP and Control groups, genotypes and alleles driven from the asses were investigated in three subgroups: AA, GA and GG. In table 3 the same comparison is performed between PI and Control.

In the tables 2 and 4 the alleles (A and G) are examined separately for respectively CP and PI individually compared to Control group. Furthermore in tables 5 & 6 the diseased samples were compared together first by genotype aspect and secondly by the alleles.

Finally in table 7 the results are shown for the gender-dependency investigation in two subgroups: obviously males and females.

Table 1. Genotype frequencies within chronic periodontitis and control groups.

Genotypes	Chronic Periodontitis	Control	Total	P-Value
	n (%)	n (%)	n (%)	
A:A	2 (50)	2 (50)	4 (100)	0.864
G:A	18 (41.9)	25 (58.1)	43 (100)	
G:G	53 (48.6)	56 (51.4)	109 (100)	

Table 2. Allele frequencies within chronic periodontitis and control groups.

Groups Allele	Chronic Periodontitis	Control	P-Value
	n (%)	n (%)	
Allele G	124 (84.9)	137 (82.5)	0.5671
Allele A	22 (15.1)	29 (17.5)	
Total	146 (100)	166 (100)	

Table 3. Genotype frequencies within Periimplantitis and control groups.

Genotypes	Periimplantitis	Control	Total	P-Value
	n (%)	n (%)	n (%)	
A:A	2 (50)	2 (50)	4 (100)	0.375
G:A	8 (24.2)	25 (75.8)	33 (100)	
G:G	27 (32.5)	56 (67.5)	83 (100)	

Table 4. Allele frequencies within periimplantitis and control groups.

Allele	Groups	Periimplantitis	Control	P-Value
		n (%)	n (%)	
Allele G		62 (83.8)	137 (82.5)	0.8116
Allele A		12 (16.2)	29 (17.5)	
Total		74 (100)	166 (100)	

Table 5. Genotype frequencies within Periimplantitis and Chronic Periodontitis.

Genotypes	Chronic Periodontitis	Periimplantitis	Total	P-Value
	n (%)	n (%)	n(%)	
A:A	2 (50)	2 (50)	4 (100)	0.635
G:A	18 (69.2)	8 (30.8)	26 (100)	
G:G	53 (66.2)	27 (33.75)	80 (100)	

Table 6. Allele frequencies within chronic periodontitis and control groups.

Allele	Chronic Periodontitis	Periimplantitis	P-Value
	n (%)	n (%)	
Allele G	124 (84.9)	62 (83.8)	0.8239
Allele A	22 (15.1)	12 (16.2)	
Total	146 (100)	74 (100)	

Table 7. Genotype and alleles frequencies within male and female groups.

Sex	Group	Genotype			P value	Allele		P value
		GG	AA	AG		G	A	
Male (N = 87)		57 (47.9)	3 (50)	26 (56.5)	0.374	140 (49.3)	35 (57.4)	0.3152
Female (N = 86)		62 (52.1)	3 (50)	20 (43.5)		144 (50.7)	26 (42.6)	
Total		119 (100)	6 (100)	46 (100)		284 (100)	61 (100)	

Discussion

In this study, we investigated whether the polymorphism of IL-17R plays a role in occurrence of periimplantitis or is just a pro-inflammatory receptor of the cytokine IL-17. Our results show that polymorphism of IL-17R does not play a role in incidence of periimplantitis.

The noticeable finding that in individuals with periimplantitis and periodontitis the expression of IL-17R allele is not meaningfully different from the healthy samples (control group) is supported by the polymorphism analysis center in KBioscience Institute (located in U.K). 83 healthy individuals were compared to 73 periodontitis-diagnosed and 37 periimplantitis-diagnosed patients. We found out that there is a not a significant difference in the prevalence of IL-17R between the groups. Although there is not a P value based differential among the mentioned groups but there

might be some points in the results worthy to be declared. As it is shown in tables 1 & 3 the distribution of AA genotype is similarly the same in all three groups: PI, CP & C. But the GA genotype is apparently lower in patients; 58.1% in Control group and 41.9% in CP group (Table 1) and also 75.8% in Control group compared to 24.2% in PI group (Table 3).

For the first time a research has been conducted on human blood samples examining the soaring-rate disease Periimplantitis and also bringing up the results next to the most common periodontal disease the chronic periodontitis. There might also be some research that are previously done using Saliva or GCF samples but not any has yet been conducted on fresh human blood samples.

Up to this exact moment the role of IL-17 in periodontal diseases is known to be controversial. While in a recent study Severino and Napimoga demonstrated

that in periodontitis patients there is an increase of IL-17 which may induce the production of other inflammatory cytokines, contributing to the pathogenesis of bone loss in some other studies (17) researchers like Ozcaka et al concluded that the salivary concentration of IL-17 was significantly lower, and that of IL-18 significantly higher, in patients from the chronic periodontitis group compared with healthy control subjects (18). Meanwhile it was suggested that IL-17 levels in GCF were nearly zero [by Pradeep AR et al, India] (19). All these finding not only added to the importance of this intriguing cytokine but also led us to use the human blood samples for the basis of investigation.

Another importance of IL-17R was pointed out in a former study investigating how helminthes could be a major selective force on subset of IL receptors containing this exact SNP of IL-17RA. In this study Matteo Fumagalli *et al.* verified that six risk alleles for inflammatory bowel (IBD) or celiac disease are significantly correlated with micropathogen richness (20).

In a recent study in 2012, an outcome of a two stage clinical research was the substantial role of Interleukin 17RA (rs879576) in never-smokers contracting lung cancer. Thus, this could remind us how inflammation-related genetic variations can affect clinical outcomes in screening the patients (21).

In this article, there was no relationship found between the IL-17R and chronic periodontitis and periimplantitis in an investigated Iranian population but further investigations on larger communities and other nationalities might lead to a clue for solving the IL-17 puzzle. One of the problems in our research was the low number of samples that has some reasons worthy to be considered for further researcher: First of all we had to filter our samples through our strict criteria and it obviously narrows the population. Secondly the low incidence of periimplantitis is an important factor and as we all know this disease is not akin to common cold catches to be investigated in large scales. Finally the blood samples were coming from human beings and the ethical view has a high importance here.

As previously mentioned, the controversy about IL-17 may not be solved easily but all these proofs like this article are likely to lead to a reasonable and final idea on IL-17R and its cytokine. This report illustrates the need for investigating a larger community of patients and also far more communities for a comprehensive conclusion. There could also be of high importance to do a similar research relating IL-17 to the aggressive form of periodontitis this time due to its strong genetic nature. It

is also necessary to suggest a complimentary test that could be added to these types of researches, which is measuring the experimented cytokine's concentrations. This action will aid us to find out what are the mean concentrations of these pro-inflammatory agents in different patients of variable communities and also assist us in a therapeutic aspect. One other limitation of our study is that our experiments have thus far been conducted only on blood samples and a more general study could put the GCF samples (if available) and saliva samples next to each other for a better comprehension.

Another subject that caught our attention was the role of gender in the incidence of periodontal disease. As it was examined thoroughly before in mice (20) we came up to another analysis in our results (Table 7) and it revealed that there is no meaningful relation between IL-17 polymorphism-role in men and women compared to each other.

It is also note-worthy to know that the differences in this group of articles might be due to different research approaches. One of them is different periimplantitis diagnosis methods and indexes and another reason could be due to implant status evaluation in different loading times. In conclusion, the enigma of IL-17 and its polymorphism-role in periodontitis and periimplantitis is yet to be investigated more carefully throughout further researches but this article demonstrates that polymorphism of IL-17R plays no significant role in incidence of Chronic Periodontitis and Periimplantitis. However, the existing controversy makes it essential to continue the research in this field examining larger population and also non-Iranian communities as well.

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