Association of Interleukin-17 polymorphism (-197G/A) in chronic and localized aggressive periodontitis

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Abstract: Interleukin 17(IL-17) is a pro-inflammatory cytokine produced mainly by Th17 cells. The present study was undertaken to investigate a possible association between IL-17 A genetic polymorphism at (-197A/G) and susceptibility to chronic and localized aggressive periodontitis (LAgP) in an Indian population. The study was carried out on 105 subjects, which included 35 LAgP patients, 35 chronic periodontitis patients and 35 healthy controls. Blood samples were drawn from the subjects and analyzed for IL-17 genetic polymorphism at (-197A/G), by using the polymerase chain reaction-restriction fragment length polymorphism method. A statistically significant difference was seen in the genotype distribution among chronic periodontitis patients, LAgP patients and healthy subjects. There was a significant difference in the distribution of alleles among chronic periodontitis patients, LAgP patients and healthy subjects. The odds ratio for A allele *versus* G allele was 5.1 between chronic periodontitis patients and healthy controls, and 5.1 between LAgp patients and healthy controls. Our study concluded that IL-17 A gene polymorphism at (-197A/G) is linked to chronic periodontitis and LAgP in Indian population. The presence of allele A in the IL-17 gene polymorphism (-197A/G) can be considered a risk factor for chronic periodontitis and LAgP.

Keywords: Chronic Periodontitis; Aggressive Periodontitis; Polymorphism, Genetic; Interleukins.

Introduction

Chronic periodontitis and aggressive periodontitis are initiated and sustained by bacterial plaque, but host defense mechanisms play an integral role in their pathogenesis. Although periodontal pathogens express a variety of tissue-eroding virulence factors, most of the damage is actually a result of the host response mechanism.[1]

Cytokines are cell-signaling molecules that aid in cell-to-cell communication in immune responses, and that stimulate the movement of cells towards sites of inflammation, infection and trauma.[2] Interleukins are a type of cytokines that were first seen to be expressed by leukocytes. Interleukin (IL) constitutes a group of cytokines with complex immunomodulatory functions, including cell proliferation, maturation, migration and adhesion, and also plays a role in immune cell differentiation and activation.[3]

Interleukin 17(IL-17) is a pro-inflammatory cytokine produced mainly by Th17 cells and also by γδTcells and neutrophils.[4] IL-17 is also referred to as IL-17A. Other proteins homologous to IL-17 have been identified and designated as IL-17B, IL-17C, IL-17 D, IL-17E and IL-17F.[5] IL-17 appears to stimulate mostly the production of cytokines that either specifically attract neutrophils to the site of an inflammation (IL-8, granulocyte chemotactic protein-2) or stimulate granulopoiesis in bone marrow (IL-6, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor). Apart from these, IL-17 appears to induce cytokines IL-1β and TNF*a* in macrophages.[5,6] IL-17 has been associated with the increased expression of the receptor activator for RANKL (receptor activator of nuclear factor kappaB ligand), the main stimulatory factor for the differentiation and activation of osteoclasts.[7]

IL-17 may play a significant role in the etiopathogenesis of periodontal disease, since it accentuates the inflammatory reaction in periodontal tissue.[8] Research has confirmed the presence of Th17 cells in the inflamed gingiva of patients with chronic periodontitis, and further analyses have found an increased level of cytokine IL-17 in GCF samples, and in the culture supernatants of gingival cells in periodontal disease.[9,10,11]

The search for genetic markers associated with both the severity and the susceptibility of periodontal disease has been receiving considerable attention lately. In particular, polymorphisms in gene encoding molecules of the host defense system have been targeted as potential genetic markers. Allelic variants of a specific gene can cause higher or lower production of the molecule.[12]

The IL-17A gene has been mapped to chromosome 6p12. IL-17 genetic polymorphism has been associated with diseases like rheumatoid arthritis (RA), asthma, ulcerative colitis and gastric cancer.[13,14,15,16] Only few studies have been conducted to investigate the association between IL-17 gene polymorphisms and chronic periodontitis, and none have investigated its association with localized aggressive periodontitis.[17,18] The aim of this study was to investigate a possible association between IL-17A genetic polymorphism at the promoter region (-197G/A) (rs2275913) and susceptibility to chronic and localized aggressive periodontitis (LAgP), by polymerase chain reaction (PCR), in a randomly sampled Indian population.

Methodology

The present study was carried out on 105 subjects who visited the outpatient Department of Periodontics, P.M.N.M. Dental College and Hospital at Bagalkot, in accordance with the Helsinki declaration of 1975, as revised in 2002. Ethical approval for the study was obtained from the Institutional Review Board of P.M.N.M. Dental College and Hospital, Bagalkot, Karnataka, India, and the approval protocol number issued was PMNMDCH/4817/2009-10.

Method of data collection

A sample of 105 unrelated subjects from the southern region of India composed the study. It was divided into three groups, based on the criteria defined by the International Workshop for Classification of Periodontal Disease.[19] Group I: 35 patients with LAgP, Group II: 35 patients with chronic periodontitis, Group III 35 subjects with healthy periodontium. The clinical parameters recorded were the simplified oral hygiene index, the gingival index (GI), the probing depth (PD) measurement using William's graduated periodontal probe, and the clinical attachment loss (CA loss).

LAgP patients showed interproximal attachment loss, affecting at least two permanent teeth, one of which was the first molar, and involving no more than two teeth, other than first molars and incisors, in addition to a rapid rate of disease progression in an otherwise healthy individual, absence of large accumulation of plaque and calculus, and a family history of aggressive periodontitis. Chronic periodontitis patients had at least 20 natural teeth and a minimum of six periodontal pockets ≥ 5 mm, or loss of attachment of ≥ 3 mm, as well as local factors correlated with the loss of periodontal structures. Healthy controls included subjects who visited the dental college for regular checkups, and demonstrated an absence of clinical and radiographic manifestations of periodontal disease. They had at least 20 teeth present and no evidence of CA loss or probing pocket depth > 3 mm. When healthy controls were recruited, age matching was done

with chronic periodontitis patients. All the patients were systemically healthy and none had received periodontal treatment for at least 6 months prior to the sampling and data recording.

Exclusion criteria

a. Current and former smokers who had smoked 100 or more cigarettes in their lifetime;
b. Systemic diseases like diabetes mellitus and HIV infection, known to influence periodontal disease;
c. Diseases of oral hard and soft tissues, except caries and periodontitis;
d. Chronic use of anti-inflammatory drugs and medication within the 3 months prior to the study;
e. Pregnant and lactating females.

All potential participants were informed of the study needs and design. Informed consent was obtained from the participating subjects.

Collection of samples

A 5 mL volume of venous blood was collected from the cubital fossa of each subject. These blood samples were sent to the laboratory in a tube containing ethylenediaminetetraacetic acid (EDTA) for DNA amplification by polymerase chain reaction (PCR).

Laboratory procedures

DNA extraction

DNA was extracted using the modified proteinase K method. About 500 μl of a blood sample was placed in a 2 mL tube and centrifuged for 10,000 rpm for 3-4 minutes. The supernatant was discarded and 500 μl of Tris-EDTA buffer was added to the sediment. The resulting solution was mixed well and centrifuged again for 10,000 rpm for 3-4 min, and the supernatant was again discarded. This step was repeated 4 times. Then, 500 μl of lysis buffer I (4 M GuSCN, 0.5% N-lauroyl sarcosine, 1 mM dithiothreitol, 25 mM sodium citrate and 40 μg of glycogen/tube) was added, the solution was centrifuged, and the supernatant was discarded. Next, 50 μl of lysis buffer II (Tris-HCL, Nonidet P-40 and Tween 20) and 5 μl of proteinase K were added to yield the final solution. This was kept in a water bath at 75°C for 2 h and then immersed in a boiling water bath for 10 min and stored at -80°C.

DNA amplification by polymerase chain reaction

The primer pair (Bioserve, Beltsville, USA) used in the study to analyze IL-17A (-197G/A) genetic polymorphism was:

Sense: IL17AF:
5'-AACAAGTAAGAATGAAAAGAGGACATGGT-3'
Antisense: IL17AR:
5'-CCCCCAATGAGGTCATAGAAGAATC-3'

The total volume of the PCR mixture was 25 μl, containing 100 ng of genomic DNA, 1 X PCR buffer (Chromous Biotech, Bangalore, India), 5 Mm of a dNTP mix, 0.5 units of Taq DNA polymerase (Chromous Biotech, Bangalore, India), 3 pmol of each primer and 2 mM of $MgCl_2$. Amplification was performed with a conventional PCR system (Palm-Cycler, Corbett Research, Sydney, Australia). The amplification cycle consisted of an initial denaturation step at 95°C for 15 minutes, a denaturation step of 35 cycles at 94°C for 30 s, an annealing step of 35 cycles at 57°C for 30 s, an extension step of 35 cycles at 72°C for 30 s, and a final extension step at 72°C for 10 minutes. The PCR products were incubated overnight at 37°C with XagI (Fermentas, Waltham, USA), and were viewed in a 6.5% polyacrylamide agarose gel electrophoresis system stained with silver.

Statistical analysis

The Chi-square test was used to analyze the genotype distribution and the allele ratio of chronic periodontitis patients, localized aggressive periodontitis patients and healthy controls, and to test for deviation of genotype distribution from Hardy-Weinberg equilibrium. Kruskal-Wallis one-way ANOVA was used to evaluate the statistical significance among the clinical parameters of the different genotypes in each group. The odds ratio was used to measure the strength of the associations between risk factors and outcome. Statistical significance was set at p-value < 0.05. STATA version 9.2 (STATA Corp. LP, College Station, USA) was used for statistical analysis of the data.

Results

The mean age of chronic periodontitis patients and LAgP patients was 37.20 ± 4.21 (age range of 26-48), and 21.23 ± 4.56 (age range of 16-28), respectively. The gender (male/female) of LAgP patients, chronic periodontitis patients and healthy subjects was 19/16, 18/17, and 19/16, respectively. There was no statistically significant difference.

The clinical parameters of 3 different genotypes (AA, AG and GG) were compared in LAgP (Table 1) and chronic periodontitis patients (Table 2) using Kruskal-Wallis one-way ANOVA. The clinical parameters included PD, CA loss, and GI. There was no significant difference among the clinical parameters of different genotypes in chronic periodontitis and LAgP patients.

Table 3 shows the distribution of genotypes in three study groups. A statistically significant difference was seen in the genotype distribution among chronic periodontitis, LAgP patients and healthy subjects, respectively. Genotype frequencies were found to be within the Hardy-Weinberg equilibrium.

Table 4 shows the distribution of allele A and G in the study groups. A statistically significant difference

Table 1. Comparison of genotypes in respect to different variables for localized aggressive periodontitis patients by Kruskal-Wallis one way ANOVA.

Variable	Summary	AA	AG	GG	Kruskal-Wallis H-value	p-value*
GI**	Mean ± SD	1.82 ± 0.21	1.88 ± 0.17	1.87 ± 0.13	1.18938	0.5517
PD*** (mm)	Mean ± SD	4.43 ± 0.55	4.24 ± 0.55	4.61 ± 0.40	2.0333	0.3618
CA loss**** (mm)	Mean ± SD	4.12 ± 0.50	3.88 ± 0.45	3.98 ± 0.66	1.5828	0.4532

*$p < 0.05$.
**Gingival Index.
***Probing Depth.
****Clinical attachment Loss.

Table 2. Comparison of genotypes in respect to different variables for chronic periodontitis patients according to Kruskal-Wallis one way ANOVA.

Variable	Summary	AA	AG	GG	Kruskal-Wallis H-value	p-value*
GI** (mm)	Mean ± SD	1.96 ± 0.29	1.89 ± 0.28	2.07 ± 0.17	1.5066	0.4708
PD*** (mm)	Mean ± SD	2.83 ± 0.49	2.67 ± 0.58	2.91 ± 0.19	0.7157	0.6991
CA loss**** (mm)	Mean ± SD	2.05 ± 0.53	1.92 ± 0.52	2.27 ± 0.36	2.0027	0.3674

*$p < 0.05$.
**Gingival Index.
***Probing Depth.
****Clinical attachment Loss.

Table 3. Distribution of study subjects according to genotype and study groups.

Genotype	LAgP*	%	CP**	%	Healthy	%	Total
AA	18	51.43	20	57.14	9	25.71	47
AG	12	34.29	8	22.86	3	8.57	23
GG	5	14.29	7	20.00	23	65.71	35

Hardy-Weinberg equilibrium p-value for [LAgP=0.68, CP=0.68, healthy subjects =0.30].
Chi-square for total subjects = 26.3732 , p=0.00003, S.
Chi-square analysis between LAgP patients and healthy controls = 19.9721, p=0.00005, S.
Chi-square analysis between CP patients and healthy controls= 14.9783, p=0.00056, S.
*Localizedaggressive periodontitis.
**Chronic periodontitis.
 NS: Not statistically significant, S: Statistically significant (p-value<0.05)

Table 4. Distribution of study subjects according to alleles and study groups.

Genotype	LAgP*	%	CP**	%	Healthy	%	Total
A	48	68.57	48	68.57	21	30.00	117
G	22	31.43	22	31.43	49	70.00	93

NS: Not statistically significant; S: Statistically significant (p-value < 0.05).
Chi-square for total subjects = 28.1391, p = 0.0000, S.
LAgP patients *versus* healthy controls [Chi-square analysis = 20.8336, Odds ratio = 5.1, p 0.0000, S].
Chronic periodontitis patients *versus* healthy controls [Chi-square analysis = 20.8336, Odds ratio = 5.1, p 0.0000, S].
*Localized aggressive periodontitis.
**Chronic periodontitis.

was seen in the distribution of alleles among chronic periodontitis patients, LAgP patients and healthy subjects. The odds ratio for A allele *versus* G allele was 5.1 between chronic periodontitis patients and healthy controls, and 5.1 between LAgP patients and healthy controls.

Discussion

The pathophysiology of periodontitis, as is also the case with other complex diseases, is characterized by various biological pathways leading to the same clinical phenomena.[20] Multiple genes and their polymorphisms may all offer a small overall contribution and pose a relative risk to disease susceptibility and severity.[21] A number of scientific papers also support the role of genes in host responses and in the progression of periodontal disease. Additional support for a genetic contribution to periodontitis emerged from the identification of certain genetic polymorphisms that correlate with immune response phenotypes in certain groups of periodontitis patients.[22]

In vitro studies have indicated a pro-inflammatory function for IL-17; it induces fibroblasts and epithelial cells to produce more IL-6, granulocyte colony-stimulating factor (G-CSF) and several chemokines. IL-17 genetic polymorphism has been found to be associated with many inflammatory diseases in humans, such as rheumatoid arthritis, asthma and ulcerative colitis.[13,14,16] According to Takahashi et al.[8] IL-17 is produced by T cells in periodontal lesions, and may exacerbate inflammatory reactions both directly and indirectly, by triggering the release of inflammatory mediators from human gingival fibroblasts in the periodontal tissues.

Humans with inherited disorders of neutrophil chemotaxis have a high frequency of localized aggressive periodontitis; moreover, genetic polymorphisms affecting chemokine expression have been associated with susceptibility to periodontal disease. Apart from its pro-inflammatory role, IL-17 plays a particularly significant role in regulating neutrophil recruitment and granulopoiesis. In bacterial infections such as periodontal disease, IL-17 may be critical for recruiting neutrophils and other immune cells required to limit the spread of infection.[23]

Many studies have reported an increased expression of IL-17 at the site of moderate to severe periodontitis.[8,9,24,25,26,27] In contrast, Pradeep et al.[28] reported a lower concentration of IL-17 in gingival crevicular fluid of periodontitis patients. Schenkein et al.[29] reported an elevated level of IL-17 in the systemic circulation of patients with localized and generalized aggressive periodontitis. The biological mechanisms behind the association between positive genotype and periodontal disease are based on the hypothesis that these polymorphisms may modulate the secretion of IL-17.[17]

Two previous studies have been conducted to analyze the association between IL-17 genetic polymorphism and periodontitis. Correa et al.[17] carried out a study to evaluate the association between chronic periodontitis and genetic polymorphism at IL-17 (-197G/A) and IL-17F (7488C/T). Another study has also been performed to analyze the association between chronic periodontitis and IL-17 genetic polymorphism (rs10484879).[18] However, no study has been done as of yet to analyze the association between IL-17 genetic polymorphism at (-197G/A) and periodontitis on an Indian population. This is probably the first study designed to evaluate the association between LAgP and IL-17 genetic polymorphism at (-197G/A).

The present study showed a significant difference in genotype distribution when all three groups were compared together. The main findings of this study

were that the carriage of AA genotype of IL-17A -197 was significantly associated with the occurrence of both chronic periodontitis and LAgP, and that genotype GG was associated with healthy periodontium. Our findings were in line with the study by Correa et al.,[17] who reported a greater incidence of AA genotype in chronic periodontitis patients. Kadkhodazadeh et al.[18] observed a positive association between the CC genotype of IL17 polymorphism (rs10484879) and chronic periodontitis.

The frequency of the IL-17A (-197A) allele was significantly greater in both chronic periodontitis and LAgP patients, as compared with healthy controls. Correa et al.[17] concluded that the IL-17A (-197A) allele is associated with an increased risk for chronic periodontitis in Brazilian population. Our findings suggest that the IL-17A (197A) allele is associated with chronic periodontitis and LAgP in Indian population. Subjects with A allele are at 5 times greater risk of developing chronic periodontitis than healthy controls (odds ratio = 5.1, confidence interval = 95%). Similarly, subjects with A allele are at 5 times greater risk of developing LAgP periodontitis than healthy controls (odds ratio = 5.1, confidence interval = 95%).

Enzyme-linked immunosorbent assay (ELISA) has proven that the amount of IL-17A in the serum of chronic periodontitis patients with allele A is greater than in patients with allele G. Moreover, a greater amount of IL-8 and myeloperoxidase activity was observed in the gingival tissues of chronic periodontitis patients with the AA and AG genotype than with the GG genotype.[17] The -197A allele of IL-17A has also been linked with inflammatory diseases such as rheumatoid arthritis and ulcerative colitis, and tumor like gastric cancer.[13,15,16]

In our study, there was no significant association between individual genotypes (AA, AG, and GG) and clinical parameters, *i.e.*, PD, CA loss, and GI, in chronic periodontitis and LAgP patients analyzed separately. This was in contrast to the study conducted by Correa et al.,[17] where a significantly higher PD and CA loss was found in patients with the AA and AG genotype, as compared with the GG genotype. However, no significant correlation was found between genotypes and bleeding on probing.

A polygenic model for periodontitis was presented by Yoshie et al.[21] It stated that aggressive periodontitis and chronic periodontitis are not monogenic but polygenic diseases. Some genetic polymorphisms are common for both chronic and aggressive periodontitis. The findings of this study suggest that genotypes AA and AG of IL-17A (-197) are more common in chronic periodontitis and LAgP patients, and that -197A allele of IL-17A is significantly associated with localized aggressive periodontitis and chronic periodontitis in Indian population.

The major limitation of the study was the comparatively small sample size for a genetic study. The reason was the inclusion of localized aggressive periodontitis patients, whose prevalence in the general population is low. However, genetic studies conducted previously with aggressive periodontitis patients used a similar sample size.[1]

Conclusion

The present study concludes that IL-17A gene polymorphism at (-197A/G) was significantly associated with localized aggressive periodontitis and chronic periodontitis in Indian population. The presence of allele A in the IL-17 gene polymorphism (-197A/G) can be considered a risk factor for the development of chronic periodontitis and localized aggressive periodontitis. Further studies need to be done involving a larger sample size and different ethnic groups to confirm the role of IL-17 genetic polymorphism (-197A/G) in chronic periodontitis and localized aggressive periodontitis.

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