Evaluation of interleukin -1B (+3954) gene polymorphism in patients with chronic and aggressive periodontitis: A genetic association study

Sujata S. Masamatti, Ashish Kumar[1] , Tarun Kumar A. Baron[2], Dhoom S. Mehta[2], Kishore Bhat[3]

Abstract

Background: IL-1 cytokines have central roles in the pathogenesis of periodontal disease. Polymorphism in the locus +3954 (C/T) of the human IL-1B gene has been shown to affect the levels of this cytokine. **Aim:** The aim of the present study was to investigate the association between the IL-1 B (+3954) gene polymorphism and the occurrence of different clinical forms of periodontitis. **Materials and Methods**: Genomic DNA was obtained from 90 individuals and amplified using the PCR with specific primers flanking the locus +3954 of IL-1B. PCR products were submitted to restriction endonuclease digestion and analyzed by gel electrophoresis, allowing for the determination of the genotypes and detection of the polymorphism. **Statistical Analysis:** Fisher's exact test was used for comparing the frequency of genotype distributions between groups. **Results:** The chronic periodontitis group displayed a higher percentage of T alleles (38%) when compared to the aggressive periodontitis group (20%) and to the control group (19%). **Conclusion:** Our study data states that polymorphism in the locus +3954 of IL-1B gene could be a risk factor for chronic periodontitis in a sample of Indian population of Karnataka state.

Keywords: Gene polymorphism, interleukin-1beta, periodontitis

Introduction

The microbial causation of the periodontal diseases is well established.[1] There are, however, other elements that influence the inflammatory and immune response both locally and systemically. These include systemic diseases such as diabetes and environmental factors such as smoking and possibly stress.[2]

In the last decade, convincing evidence has emerged that susceptibility to microbial infections may be determined in part by the host's genotype and there exists a heritable component to periodontitis.[3] There are several features of the host's innate and acquired immune systems that have some heritable element and may contribute to genetic susceptibility to periodontitis.[3]

A large body of work from *in vivo* and *in vitro* analyses of human tissues as well as studies in animal models strongly supports the notion that cytokines play a key role at all stages of the immune response in periodontal disease[4] Cytokines are potent immunoregulatory molecules serving as potential diagnostic markers of periodontal disease.[4]

While several immune mediators influence the development of tissue inflammatory responses, interleukin-1 is likely to be a major cytokine involved in most inflammatory responses. Interleukin-1 is a proinflammatory cytokine that has been implicated in mediating acute and chronic inflammatory diseases and is produced mainly by stimulated monocytes, macrophages, keratinocytes, smooth muscle, and endothelial cells.[5] Furthermore, interleukin-1 triggers enzymes leading to the production of prostaglandin E2 (PGE2) and is a primary regulator of matrix metallo-proteinases (MMPs) and their inhibitors. Importantly for periodontal disease, interleukin-1 also is known to be one of the most active stimulators of osteoclastic activity.[6]

The interleukin-1 family consists of three homologous proteins; interleukin-1 α and interleukin-1β, which are pro-inflammatory proteins, and interleukin-1ra, an antagonist protein. These proteins are encoded on chromosome 2q13-21 and are polymorphic at several loci.[7]

Single nucleotide polymorphisms in the interleukin-1 locus, their functional consequences, and their association with susceptibility to and severity of various chronic inflammatory diseases have been described in the literature.[8] Some reports

Department of Periodontics, I.T.S-CDSR, Murad Nagar, [1]*Institute of Dental Studies and Technologies, Modinagar, Ghaziabad, Uttar Pradesh,* [2]*Periodontology and Implantology, Bapuji Dental College and Hospital, Davangere,* [3]*Microbiology, Maratha Mandal Dental College and Research Centre, Belgaum, Karnataka, India*

Correspondence: Dr. Sujata S Masamatti, 24 DDA Flats, Taimoor Enclave, New Friends Colony, New Delhi - 110 065, India.
E-mail: sujata_sm10@yahoo.com

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indicate that polymorphisms in the IL-1 gene cluster may influence the variations in the synthesis of cytokines, and thus modify the individual responses to bacterial stimuli.[9]

With regard to the interleukin-1 polymorphism, it has been suggested that a haplotype comprising at least one single nucleotide polymorphism in each of the genes encoding the interleukins IL-1α and IL-1β increases the susceptibility for periodontal diseases.[9]

Considering the frequency of many allele variation between ethnic groups and geographically distinct populations, and that studies concerning IL-1B gene polymorphism and different forms of periodontitis are not many; particularly from India, the purpose of the present study was to evaluate the association between the IL-1B (+3954) gene polymorphism and different clinical forms of periodontitis involving individuals from the state of Karnataka in south Indian population.

Materials and Methods

The present study employed a case-control design involving subjects visiting the Out Patient Department of Periodontics, Bapuji Dental College and Hospital, Davangere, Karnataka. India.

Study sample included subjects belonging specifically to Karnataka state only. A total number of 90 patients were included in this study, with the age of the patients ranging from 15 to 70 years including both the sexes. The patients were divided in to three groups, composed of 30 subjects each, involving patients with aggressive periodontitis (group A), chronic periodontitis (group B), and a healthy control group (group C) (AAP 1999).[10] The subjects included in the study were in good general medical health. Subjects were excluded from the study if they had any systemic disease, bleeding disorders, or immunosuppressive diseases. Pregnant and lactating mothers, subjects under any anti-inflammatory and/or immunosuppressive drugs, subjects who were tobacco users (whether in a smoking and/or smokeless form) were also excluded.

The research protocol was approved by the local ethical committee before starting the study.

A complete clinical examination was carried out. A mouth mirror and a University of Michigan 'O' probe [with William's markings (Hu-Friedy, USA)] were used to assess periodontal findings. Simplified oral hygiene index (OHIs),[11] clinical attachment level (CAL), and Russel's Periodontal Index[12] were utilized for clinical examination. The medical and personal histories of patients, including oral hygiene habits were recorded.

An informed consent was signed by each patient after providing detailed explanation about the study design.

Procedures

Epithelial cells were obtained through an oral swab performed with a sterile plastic spatula and immersed in a tube containing Kreb's buffer. Within 24 h of sample collection, samples were sent to the laboratory of microbiology department. Genomic DNA was extracted from the sample.[13] PCR[14] was carried out in a total volume of 50 μl reaction mixture, containing 10 μl of solution, DNA pre-mix buffer, and primers (20 pmol/reaction) [Table 1].

Primer sequence[15] used in the reaction was forward primer: 5′-CTC AGG TGT CCT CGA AGA AAT CAA-3′; Reverse primer: 5′-GCT TTTT TTG CTG TGA GTC CCG-3′. Thermal cycling conditions were 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 35 s and extension of 72°C for 30 s. The run was terminated by final elongation at 72°C for 5 min.

Amplification was performed in a PTC-100-60 thermocycler (Corbett Research Germany). The horizontal gel electrophoresis[16] system was employed for the detection of total DNA. Gels were stained with ethidium bromide (0.5ug/ml), and DNA was visualized under UV (ultraviolet) light (Transilluminator).

Restriction fragment length polymorphism analysis

Samples that were positive for IL-IB genotypes were subjected to restriction fragment length polymorphism (RFLP). Here, the DNA that was amplified by PCR was digested by restriction enzyme *TaqI (5 U)* at 65°C to obtain the allele distribution. Resultant RFLP products were electrophoresed at 120 V on a 2.2% agarose gel stained with ethidium bromide.

Digestion of PCR product with TaqI, the restriction enzyme, lead to 97+85+12 bp fragment referred to as allele1 [C] and a 182 +12bp fragment referred to as allele2 [T]. An uncut fragment present was heterozygous and was referred to as CT.

Data analysis

Statistical analysis was performed using the SPSS (statistics packaged for social sciences) software. Fisher's exact test was used for comparing the frequency of genotype distributions between groups. Odds ratio was determined to assess the strength of relationship between genotypes and diseased groups. A *P* value of 0.05 or less was set for significance. The chi-square test was utilized to test for deviation of genotype frequencies from Hardy Weinberg equilibrium expectations.

Results

The characteristics of the study groups are mentioned in Table 2. The mean values of OHI-S were compared between groups by utilizing unpaired Student '*t*' test. When the aggressive periodontitis group (A) was compared with the chronic periodontitis group (B), a value of 1.59 was

Table 1: Genotyping of the polymorphic variant at IL-1 B+3954

Primer sequence (5′à 3′)	PCRSize (bp)		Digestion	
Forward primer: 5′-CTC AGG TGT CCT CGA AGA AAT CAAA-3′, Reverse primer: 5′-GCT TTT TTG CTG TGA GTC CCG-3′	Size (bp)	Annealing temp.	Enzyme	Incubation temperature
	182 bp	54°C	Taq1	65°C

Table 2: Characteristics of the Study Groups

	Aggressive periodontitis (Group A)	Chronic periodontitis (Group B)	Control (Group C)
No of individuals (*n*)	30	30	30
Age range (year) (Mean ±SD)	29.08 ± 5.7.	44.4 ± 7.6	26 ± 4.7
Gender Male (%) Female (%)	 14 (46.66) 16 (53.33)	 20 (66.66) 10 (33.33)	 10 (33.33) 20 (66.66)
OHI-S (Mean ± SD)	2.58 ± 0.50.	2.89 ± 0.94	1.12 ± 0.58
Clinical attachment loss (mm)	5.70 ± 1.30	5.18 ± 0.81	0
Russel's periodontal index (P.I)	5.52 ± 0.95	5.26 ± 0.78	1

Table 3: intergroup comparison of genotype distribution by fishers exact test

	Groups compared	*P* Value	OR
CC	A vs. B	0.20	1.9
	C vs. B	0.07	2.5
TT	B vs. A	0.05*	9.2
	C vs. A	0.34	2.3
	B vs. C	0.08	3.9
CT	A vs. B	0.58	1.3
	A vs. C	0.26	1.8
	B vs. C	0.55	1.3

* Statistically significant

obtained, which was statistically insignificant (P=0.12). A statistically significant value was obtained when the Aggressive Peridontitis (A) group was compared with the control group (C) which was 10.5 with a P value 0.01. Further the chronic periodontitis group (B) and the control group (C) were compared which gave a statistically significant value of 8.82 (P = 0.01).

The mean probing pocket[17] depths for group A and B were 5.51±1.0 and 4.29 ± 0.70. On comparison of groups we obtained a statistically significant value 3.89 (P = 0.01). The

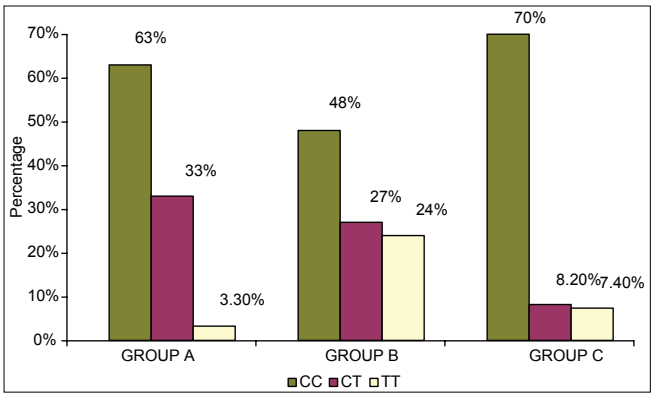

Figure 1: Distribution of the Il-1 B (+3954) Genotype in the Study Groups

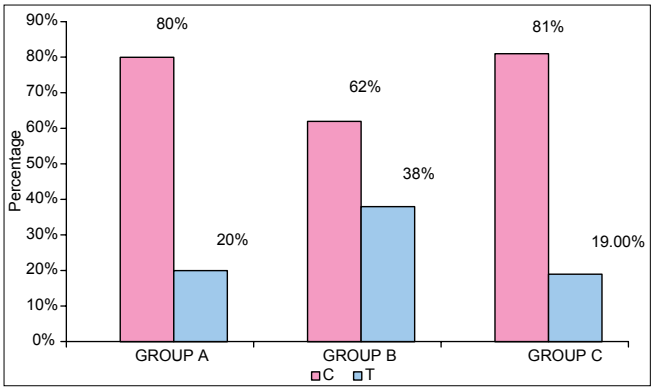

Figure 2: Distributions of alleles in the study groups

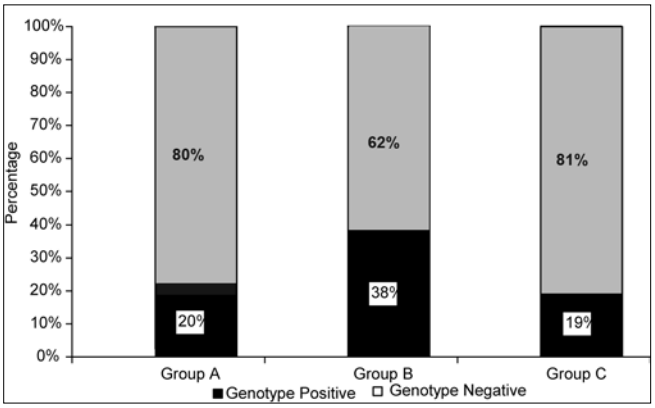

Figure 3: Analysis of T+ and T- genotype in the study groups

mean clinical attachment loss in aggressive periodontitis was 5.70 ± 1.30 and in chronic periodontitis it was 5.18 ± 0.81. On comparison, no statistical significant result was obtained (P=0.07).

The Russel's Periodontal Index score for Group A was 5.52 ± 0.95 and for Group B it was 5.26 ± 0.78. On comparison a value of 1.16 (P=0.25) was obtained which was not statistically significant.

The homozygous for the allele 1 i.e. CC of the IL-1B +3954 was the most frequent genotype in cases and controls [Figure 1]. When the distribution of this dominant genotype (i.e. CC) was compared between groups A, B, and C, statistically insignificant values were obtained. (A vs B; P = 0.20, OR 1.9. B vs C; P = 0.07; OR 2.5).

The heterozygous genotype (CT) dominated in cases than in controls. When the distribution of this CT allele was compared among groups [Table 3], we obtained a statistically insignificant values (P = 0.58 for group A vs. B, 0.26 for group A vs. C and 0.55 for group B vs. C).

The prevalence of the TT genotype was significantly higher in the chronic periodontitis group (24%) than in both aggressive and control groups (3.3% and 7.4%). When we compared the chronic periodontitis group with the aggressive periodontitis group [Table 3], a statistically significant value was obtained with a P value being 0.05 (OR=9.2). No statistical difference was observed on comparing Control and aggressive periodontitis patients groups (P=0.34).

The presence of T allele (allele 2) in a group was referred to as T+ (genotype positive) and the absence of T allele was referred to as T– (genotype negative). Intergroup comparisons [Figures 2, 3] of T+ and T– were made by utilizing the Fisher's exact test. When the groups A and B were compared a statistically significant value was obtained (P=0.05).

Comparison of observed genotype distribution to Hardy Weinberg equilibrium

In the present study, no differences were found between observed and expected distributions of genotypes for the aggressive periodontitis group. However, the genotype frequencies of the chronic periodontitis and control groups deviated from that of Hardy-Weinberg Equilibrium proportion. But the deviations were not statistically significant.

Discussion

In the present case control study, subjects included were belonging to Karnataka state only. This is due to the fact that prevalence of IL-1 genotype vary with different ethnic and racial groups, further findings of one ethnic population cannot be extrapolated to other.[18]

Individuals suffering from bleeding disorders, immunosuppressive diseases, or systemic diseases such as diabetes mellitus were excluded from the study because their inclusion would affect the distribution of samples and may interfere with the association of periodontal disease with genotype status. Smokers and smokeless tobacco users were excluded from the study because smoking[2] has been established as a major risk factor in the development of periodontal disease. The association between composite genotype and the advanced form of periodontitis in a study was significant only when smokers were excluded from that study.[9]

Cases had a mean age slightly higher than controls. In many periodontal studies, ages matching between patients and controls have not been considered necessary because the genetic patterns don't change with age.[19]

Composite genotype as stated by Kornman[8,9] referred to the presence of specific polymorphism, allele 2 at IL-1A -889 and IL-1B +3953 loci. Currently, genetic tests assess for the presence of at least one copy of allele 2 at the IL-1A +4845 and at least one copy of allele 2 at the IL-1B+3954 locus.

The IL-1B+3953 polymorphism has been renumbered and is now referred to as the IL-1B+3954 polymorphism. This occurred because current convention indicates that the numbering of the transcription should begin at +1 instead of zero.[20]

The homozygous genotype (CC) of IL-1B +3954 was the most frequent genotype in control subjects than in cases, where as the heterozygous (CT) genotype dominated in cases than in controls. This finding is in accordance with studies conducted in Brazil,[21] Poland,[22] and Chile[23] as well as in Germany.[24] However, contradictory results were obtained by studies conducted in Iran[25] and in a recently published data[26] where they have found that CC genotype was the more frequently found genotype in cases than in controls.

The prevalence of the TT genotype of the IL-1B (+3954) was significantly higher in the chronic periodontitis group (24%) than in both aggressive and control groups, 3.3% and 7.4%, respectively. In group A 20% of subjects were T+ and 80% were T–. In group B, 38% were T+ and 62% were T–. In group C, 19% were T+ and 81% were T–. Statistically significant values were obtained when group A was compared with group B (P=0.05).

This finding is in accordance with a study where the investigators observed that the IL-1 B +3954 polymorphism alone was associated with chronic periodontitis in Caucasian patients resident in Western Australia.[27] Also, our study results matched with studies, where an observed association of IL-1 B +3954 with chronic periodontitis in Brazilian[21] and Indian population[28,29] was present. Conversely, there are reports in which the investigated polymorphism was not associated with chronic periodontitis.[26,30]

With regards to aggressive periodontitis and the gene polymorphism, no association was observed in our study. Our

results are in accordance with studies,[25,27] where there exists no significant differences in frequencies of the alleles between aggressive periodontitis patients and the control group.

However, contradictory data were reported by Parkhill *et al*[31] who observed an increased frequency of the C allele in aggressive periodontitis of Caucasian population when compared with the control group. Also a study conducted in Turkish[32] population revealed a significant association of Il-1B gene polymorphism and aggressive periodontitis individuals.

One plausible interpretation for the association of the IL-1 B gene polymorphism and periodontal disease is based on the fact that the evaluated polymorphism has been described as a functional polymorphism. Thus, the polymorphic genotypes would directly influence the disease pathogenesis via an effect on cytokine synthesis. An exacerbated expression of IL-1β could lead to higher inflammation and tissue destruction. However, results observed in various studies reflect differences in the role of IL-1 B polymorphism in pathogenesis of aggressive periodontitis, further suggesting that genetic differences may be related to different types of periodontal diseases.

In the present study, 19% of healthy subjects were positive for IL-1B allele 2 as against 38% in the chronic periodontitis group. This finding can be interpreted in two ways: (1) IL-1B allele 2 is significantly over represented in the chronic periodontitis group, (2) the finding that 19% of healthy subjects also were positive for IL-1B allele 2 reinforces the fact that a risk factor can be present without always being associated with clinical disease.

The 38% occurrence of the positive genotype in chronic periodontitis found in this study is less than 40.6% rate in Australians of essential European heritage.[33] However, it was significantly more than 28% found in Brazilian[21] individuals, 23.33% in a recent publication in Indian[28] population. However, it is becoming increasingly clear that such rates vary greatly between ethnically and racially distinct populations.

The applied genetic study determines the presence of a specific genetic polymorphism that would contribute to further advancement of the disease in the presence of local etiological factors. This polymorphism is related to the severity of periodontal disease but not with its initiation. Thus, odds ratio (O.R) was calculated from a number of subjects positive for genotypes in aggressive periodontitis and chronic periodontitis groups rather than from healthy subjects. The greatest risk for developing periodontitis was seen in subjects positive for genotype (O.R=9.2).

Limitations and short comings of the present study

1. The results of the present study would have been more authentic if quantitative detection of IL-1β cytokine levels would have been done in GCF samples of genotype-positive and genotype-negative subjects. However, due to the higher cost of ELISA kits used for the same and other technical problems, IL-1β cytokine levels were not detected.
2. We could have analyzed the other interleukin 1 polymorphisms such as IL-1A (-889), IL-1 RN VNTR along with the IL-1B genotype. It is possible that other genetic polymorphisms may act synergistically (TNF-α 308 allele 1, RAGE 1704 G/T and FcγRIIIb2).

Conclusion

In conclusion, our study clearly demonstrated that the IL-1B+3954 gene polymorphism is associated with chronic periodontitis in Indian population of Karnataka ethnicity. The observation, that the frequency of its occurrence was virtually identical in the control group of periodontally healthy subjects casts doubt on its usefulness as a screening tool of susceptibility to periodontal infections. At the same time, it is important to realize that knowledge of the IL-1 genotype of a given patient may still prove to be useful, if longitudinal data of disease progression or therapeutic outcomes reveal that genotype-positive subjects have a poorer prognosis.

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