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Full Length Research Paper

Interleukin-1b gene polymorphism and its association with chronic periodontitis in South Indian population

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Interleukin-1 beta (IL-1b) is a potent inflammatory mediator and an important polymorphism in the locus +3954 (C/T) of the human IL1B gene has been shown to affect the levels of this cytokine. This functional polymorphism has been associated with the establishment of inflammatory diseases, including periodontal disease, in European, Asian and North American populations. The aim of this study was to investigate the association between the IL1B (+3954) gene polymorphism and the occurrence of different clinical forms of periodontitis in South Indian population. This study employed a cross-sectional design involving individuals from the State of Tamil Nadu in the Southern region of India. Genomic DNA was obtained from 5 ml of venous blood and amplified using the polymerase chain reaction (PCR) with specific primers flanking the locus +3954 of IL1B. PCR products were submitted to restriction endonuclease digestion and analyzed by polyacrylamide gel electrophoresis, to distinguish alleles T and C of the IL1B gene, allowing for the determination of the genotypes and detection of the polymorphism. The chronic periodontitis group displayed a higher percentage of the T allele (28%) than C allele (8.7%). Our data suggested that the polymorphism in the locus +3954 of IL1B gene could be a risk factor for chronic periodontitis in South Indian population.

Key words: Gene polymorphism, interleukin 1 beta, periodontitis, allele.

INTRODUCTION

Periodontal disease is an inflammatory illness that represents the main cause of tooth loss in developed countries, with increasing prevalence in the developing world (Albandar and Rams, 2000). Although the presence of gram-negative bacteria is essential for initiating and perpetuating periodontal disease, environmental as well as genetic factors contribute to individual variations in the etiology and course of disease (McDevitt et al., 2000). Studies in twins have indicated that a substantial portion of this inter-individual variability of periodontal parameters may be attributable to genetic factors (Michalowicz et al., 1991, 1994). Several reports indicate that polymorphisms in the interleukin-1 beta (IL-1b) gene cluster may influence this variation and thus, the severity of periodontal disease (Kornman et al., 1997; Parkhill et al., 2000; Gore et al., 1998). IL-1 is a pro-inflammatory cytokine that plays a pivotal role in several chronic diseases. This cytokine is a primary activator of early chemotactic cytokines, as well as of the expression of adhesion molecules that facilitate migration of leucocytes into tissues. IL-1 is also known to be one of the most active stimulators of osteoclastic bone reabsorption (Lang et al., 2000).

There are three genes that regulate the production of IL-1: IL1A, IL1B and IL1RN (Nicklin et al., 1994). These genes are located on chromosome 2q13. Genes IL1A and IL1B control the production of the pro-inflammatory proteins, IL-1a and IL-1b, respectively. IL1RN controls the synthesis of an antagonist protein (IL-ra) (Greenstein and Hart, 2002). Polymorphism in the +3954 locus (due to nomenclature change, the polymorphism at IL1B +3953 is now referred to as IL1B +3954 10), of the IL1B gene has been associated with an increased production of this cytokine. Homozygous individuals for the T allele produce a four-fold higher amount of IL-1b compared to individuals displaying the CC genotype (Pociot et al., 1992). It has recently been suggested that this polymorphism may explain why some people have a more vigorous response than others to the same stimulus (Lang et al., 2000). Several studies have evaluated gene polymorphisms in individuals with periodontitis in distinct

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Table 1. Characteristics of the study groups.

Clinical forms	Chronic periodontitis	Healthy control
Males	12	29
Females	13	21
Cal / PD (mm)	1.8 ± 0.4	5.33 ± 1.12
Current smokers	-	10
Former smokers	2	15
Non smokers	23	25

populations. Kornman et al. (1997) demonstrated that the occurrence of IL1A (–889) and\ IL1B (+3954) polymorphisms simultaneously was associated with a severity of chronic periodontitis in non-smoker Caucasians. Walker et al. (2000) observed a high prevalence of IL1B (+3954) allele C in the African-American population and concluded that this polymorphism would provide little diagnostic or predictive information for localized aggressive periodontitis.

As the frequency of many genetic alleles varies between ethnic groups and because contradictory results may be observed (10), the aim of this study was to describe the prevalence of the IL1B (+3954) gene polymorphism in South Indian population with chronic periodontitis.

MATERIALS AND METHODS

Patients

The study employed a cross-sectional design involving individuals from the State of Tamil Nadu in the Southern part of India. A total of 75 patients receiving treatment in SRMC dental college and hospital were included in this study. The patients were stratified into two groups: subjects with chronic periodontitis (n ¼ 50) and healthy volunteers as the control group (n ¼ 25). Patients in the chronic periodontitis group were 27 – 67 years old and exhibited inflammation, loss of clinical attachment due to destruction of periodontal ligament and loss of the adjacent supporting bone. All patients in the chronic periodontitis group had at least three teeth exhibiting sites of clinical attachment loss in at least two different quadrants. Diagnosis of disease was made considering the patient's medical and dental histories, radiographic findings and observations of clinical signs and parameters, including probing depth, assessment of clinical attachment loss, observation of tooth mobility, bleeding on probing and presence of plaque/calculus. Measurements of probing depth and attachment level were assessed at six locations around each tooth. The severity of disease was characterized on the basis of the amount of clinical attachment loss, within each clinical form. Patients exhibiting clinical attachment loss ‡ 5 mm were considered with severe periodontitis and those exhibiting clinical attachment loss ‡ 3 to < 5 mm were considered with moderate periodontitis. Clinical diagnosis and determination of disease severity were based on criteria established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions (Armitage, 1999). The control healthy individuals included in the study were 21 – 70 years old and did not have disease at the time of sample collection and also did not present a history of previous periodontal disease; as determined by absence of clinical attachment loss and no sites with probing depth > 3 mm.

A questionnaire was applied to all individuals enrolled in this study, in order to obtain information regarding dental history, family history of periodontal disease, smoking habit, as well as general health concerns. Use of orthodontic appliances, chronic usage of anti-inflammatory drugs, history of diabetes, hepatitis or HIV infection, immunosupressive chemotherapy, bleeding disorders, severely compromised immune function, pregnancy or lactation were regarded as exclusion criteria. Except for the presence of periodontitis, the patients included in this study were systemically healthy. Since tobacco smoking is an important risk factor for periodontitis, we also analyzed our data taking the habit of smoking into consideration. Smokers were defined as current smokers/former smokers (more than 10 cigarettes/day) and non-smokers included individuals that had never smoked. Individual consent form was obtained from all the patients and ethical committee approval was obtained before the beginning of collection of samples.

Sample collection and DNA extraction

5 ml of venous blood was collected in a sterile tube with a pinch of EDTA in it. DNA extraction was performed as described by Boom et al. (1990) and modified as follows. The supernatant was removed and 20 ll of silica (SiO2, Sigma, St Louis,\ MO, USA) and 450 ll of lysis buffer (6.0 M GuSCN, 65 mM Tris-HCl pH 6.4, 25 mM EDTA and 1.5% Triton X- 100) were added to the microtubes. Samples were homogenized by using a vortex and incubated for 30 min at 56°C. After this incubation, samples were submitted to another centrifugation and the supernatant was discharged. The pellet obtained (with DNA adsorbed on the silica) was washed twice with 450 ll of washing buffer (6.0 M GuSCN, 65 mM Tris-HCl pH 6.4), twice with 450 ll of 70% ethanol, once with 450 ll acetone and dried at 56°C for 20 min. Finally, 100 ll of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added and incubated at 56°C for 12 h to release the DNA. After incubation, the solution was homogenized, centrifuged and the supernatant containing DNA transferred to a new tube. Polymerase chain reaction and restriction endonuclease digestion IL1B (+3954) polymorphisms were assessed by polymerase chain reaction (PCR) amplification and digestion. The sequences of PCR primers were 5□-CTCAGGTGTCCTCGAAGAAATCAAA-3¢ and 5¢-GCTTTTTTGCTGTGAGTCCCG-3¢ with expected PCR product size of 194 bp, as described previously (Kornman et al., 1997). PCR was carried out in a total volume of 50 ll, containing 10 ll of solution DNA, Pre-mix buffer (50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.1% Triton X-100, 1.5 mM MgCl2, deoxynucleoside triphosphates, Taq DNA polymerase) and primers (20 pmol/reaction). The amplification conditions consisted of 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 54°C for 35 s and 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 (MJ Research, Waltman, MA, USA). The products were digested with 5 U of TaqI at 65°C for 4 h and obtained 97 + 85 + 12 bp DNA products for

allele C and 182 + 12 bp DNA products for allele T. The visualization was performed in a 10% polyacrylamide gel electrophoresis and the statistical analysis of data was also performed. The chi-squared test was used to compare the genotypes distributions between control and chronic periodontitis groups, [3· 2 contingency table, degrees of freedom (d.f.) ¼ 2). The C/T allele and T+/T genotype distribution between control and chronic periodontitis groups, multivariate logistic regression was utilized to assess the relationship of genotype to disease status while adjusting for potential confounders, such as smoking history. To exclude the possible confounding effect of smoking, in a second analysis we excluded smokers from all the different clinical groups. A p-value < 0.05 was considered significant.

RESULTS

The genotype and allele distributions of the IL1B (+3954) polymorphism are shown in Tables 2 and 3, respectively. The evaluation of the genotype distribution was performed comparing periodontal patients and individuals without disease, considering or not the habit of smoking. There was a significant difference in the genotype distribution when comparing the control group with the chronic periodontitis group (v2 ¼ 8.13, p ¼ 0.01), considering only nonsmokers (Table 2). The data indicate that the frequencies of CT and TT genotypes may be related to chronic periodontitis in non-smokers. With regards to allele distribution, statistical differences were observed when comparing control and chronic periodontitis groups (v2 ¼ 7.11, p ¼ 0.007, OR ¼ 0.24, CI ¼ 0.08–0.73), (Table 3). The chronic periodontitis group displayed a higher percentage of the T allele (28%) when compared to control (8.7%) groups. When smokers were included, a statistical difference in allele distribution and a higher percentage of T allele frequency were still observed in the chronic periodontitis group when compared to control groups (v2 ¼ 4.99, p ¼ 0.02, OR ¼ 0.26, CI ¼ 0.07–0.88) (Figure 1). The analysis of the frequency of T + individuals vs. T– individuals (in other words, the presence of T allele) between the groups was significantly different when analyzing non-smokers. These data are shown in Figure 1 and demonstrate the association of the T+ genotype with the chronic periodontitis group in non-smokers (Figure 1).

DISCUSSION

The observation that genetic polymorphisms may be associated with the establishment of distinct immune responses to microbial infections has enhanced the interest in identifying gene polymorphisms associated with diseases (Walker et al., 2000). Many studies have reported associations between cytokine gene polymerphisms and periodontitis in distinct populations (Kornman et al., 1997; Gore et al., 1998; Trevilatto et al., 2003). The frequency of many genetic alleles varies between ethnic groups and several studies have found contradictory results when comparing among distinct populations (Greenstein and Hart, 2002). Thus, the analysis of gene polymorphisms in a sample of the South Indian population represents an important advance in the study of periodontal disease in India. In this study, the organization of the sample of South Indian individuals into ethnic groups was not performed due to the strong miscegenation among Indians. Moreover, the individuals analyzed in our study, from Tamil Nadu State, are as representative of the Indian population as possible, as shown by Jaikumar et al. (Parra et al., 2003). In this study, we evaluated a polymorphism in the locus +3954 (C/T) of IL1B gene in a sample of the Indian population suffering or not from periodontal disease and found an association between the occurrence of the polymorphism and chronic periodontitis. This finding is in accordance with Rogers et al. (2002), who observed that the IL1B (+3954) polymorphism alone was associated with chronic perio-dontitis in Caucasian patients resident in Western Australia. However, these authors did not report any relationship with the habit of smoking in the analyzed patient population. Our results are in accordance with Hodge et al. (2001) and Tai et al. (2002), who observed no significant differences in frequencies of the alleles between chronic periodontitis patients and the control group in European Caucasian and in Japanese individuals, respectively. However, contradictory data were reported by Parkhill et al. (2000), who observed an increased frequency of the C allele in aggressive periodontitis in Caucasian patients when compared with the control group. The IL1B (+3954) polymorphism is widely studied considering the severity of periodontitis. Kornman et al. (1997) demonstrated an association of positive genotypes [occurrence of IL1A (– 889) and IL1B (+3954) polymorphisms simultaneously] with severe chronic periodontitis in non-smoker Caucasian patients, and McDevitt et al. (2000) reported that non-smokers or former light smokers, as defined by the authors, who displayed positive genotypes were more likely to have moderate severe periodontitis. It has been mentioned that genetic polymorphisms likely influence susceptibility to periodontitis and its clinical manifestations through the accumulated effect of multiple polymorphisms (2002). However, single polymorphisms have been associated with severity of periodontitis, as reported by Gore et al. (1998), who observed that frequencies of the T allele of the IL1B (+3954) polymorphism in Caucasians was significantly increased in severe periodontitis patients as compared to mild periodontitis patients, but not increased as compared to healthy individuals(Suzuki et al., 2004). It is a consensus in the literature concerning periodontitis that smoking is an important risk factor for the establish-ment of the disease. However, studies have shown that smokers with periodontitis have less clinical inflammation and gingival bleeding compared with non-smokers (Salvi et al., 2000). This may be explained by the fact that smoke byproducts exert local vasoconstriction, reducing blood flow, edema and clinical signs of inflammation (Salvi et al., 2000). In this study, we observed that the genetic association with

Table 2. Distribution of the IL1B (+3954) genotypes.

Genotype	Control	Chronic periodontitis
	Non smokers	
CC%	19 (82.76)	13 (51)
CT%	4 (17.24)	10 (46)
TT%		2 (3)
	Smokers	
CC%	20 (80)	25 (50)
CT%	5 (20)	20 (40.2)
TT%		5 (9.8)

Statistical difference (3 · 2 contingency table): non-smokers (control vs. chronic periodontitis): v2 ¼ 8.13, p ¼ 0.01; Results of multiple logistic regression analysis did not show a significant difference among the groups when the smokers were included.

Table 3. Distribution of IL1B (+3954) allele in control and chronic periodontitis.

Allele	Control	Chronic periodontitis
	Non smokers	
C%	41 (91.3)	36(72)
T%	5 (8.7)	14(28)
	Smokers	
C%	43 (88.6)	75(75)
T%	7 (11.4)	25(25)

The chronic periodontitis group displayed a higher percentage of the T allele (28%) when compared to control (8.7%) groups. When smokers were included, a statistical difference in allele distribution and a higher percentage of T allele frequency were still observed in the chronic periodontitis group when compared to control groups (v2 ¼ 4.99, p ¼ 0.02, OR ¼ 0.26, CI ¼ 0.07–0.88).

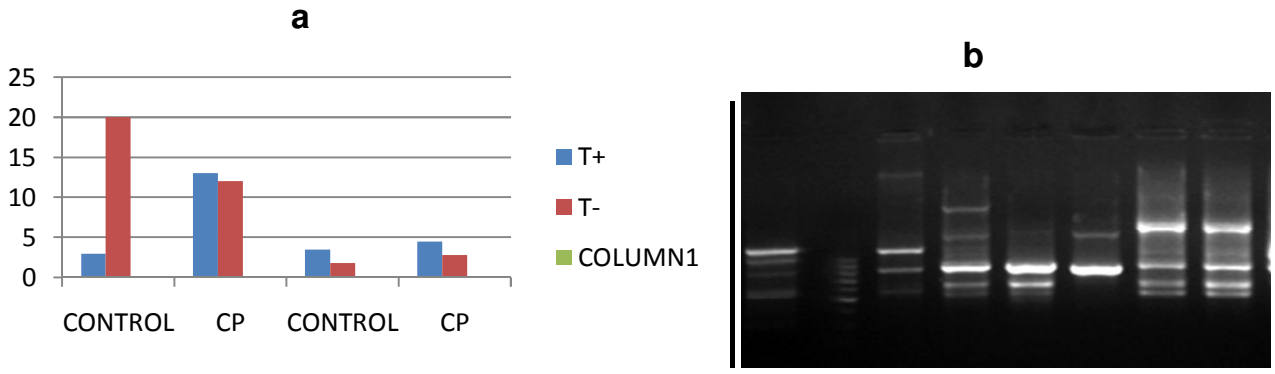

Figure 1. (a) Analysis of t+ genotype versus t- genotype. No difference was observed when smokers were included. With regards to the chronic periodontitis group. (b) 3% agarose gel electrophoresis of taqi digested for PCR amplification lanes and expression of CC (homozygous normal), CT (heterozygous mutant) and TT (homozygous mutant).

association with the chronic periodontitis was more evident when smokers were excluded from the study confirming the importance of this risk factor and suggesting that its effect is strong even in subjects who are not genetically susceptible to disease. This data suggests that the smoking- related risk can often obscure the polymorphism-related risk, as described by Kornman et al. (1997).

One plausible interpretation for the association of the IL1B gene polymorphism and periodontal disease is based on the fact that the evaluated polymorphism has been described as a functional polymorphism (Pociot et

al. 1992). Thus, the polymorphic genotypes would directly influence the disease pathogenesis via an effect on cytokine synthesis. An exacerbated expression of IL-1 could lead to higher inflammation and tissue destruction. However, results observed in various studies reflect differences in the role of IL1B polymorphism in pathogenesis of aggressive and chronic periodontitis, further suggesting that genetic differences may be related to different types of periodontal diseases (Greenstein and Hart, 2002; Walker et al., 2000). In our study, one possible limitation consists of the fact that some of the individuals who were classified, at the time of our study, as healthy might develop signs of periodontal disease in the future. However, based on the incidence of periodontal disease in India (about 10%), we would expect, at most, three individuals in our control group to develop chronic periodontitis. Thus, these individuals could bias our study. However, the loss of three individuals from our control group would not invalidate our findings. If anything, the loss of three individuals would strengthen the observed association. Finally, in many periodontal studies, age-matched studies between patients and control have not been considered necessary because the genetic patterns do not change with age (Kobayashi et al., 1997; Choi et al., 1996).

Conclusion

The present study shows that the polymorphism in the locus +3954 (C/T) of IL1B gene could be a risk factor for chronic periodontitis in the South Indian population. We believe that the identification of genetic markers for susceptibility to periodontitis will allow an early identification of individuals with high risk and could eventually help through individualized forms of therapy.

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