Study of the association between the interleukin-1 β c.3954C>T polymorphism and periodontitis in a population sample from Bahia, Brazil

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Abstract

Background: Periodontitis is an inflammatory disease characterized by the loss of connective tissue and alveolar bone. Different factors are associated with the onset and prognosis of this disease, both environmental and genetic. The latter particularly relate to molecules secreted as a function of the host immune response, such as pro-inflammatory cytokines. Studies indicate that the polymorphism c. 3954C > T in the interleukin-1 β encoding gene (*IL1B*) can be considered as an aggravating factor in the periodontitis condition. **Aims:** This study aimed to evaluate whether there is an association between the *IL1B* c. 3954C > T gene polymorphism and the prevalence of periodontitis in the population from Vitória da Conquista–Bahia, Brazil. **Materials and Methods:** A total of 347 subjects (134 cases and 213 controls) who provided epithelial tissue of the oral cavity and saliva samples for DNA extraction and quantification of *IL1B*, respectively, were selected. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism followed by electrophoresis in agarose gel. The evaluation of the cytokine concentration was performed by enzyme-linked immunosorbent assay. **Statistical Analysis:** Statistical calculations involved in this work include Chi-square test, Fisher Exact test, Mann–Whitney and Kruskal–Wallis tests. **Results:** Our findings revealed that: (i) No statistically significant relationship between periodontitis and the polymorphism studied was observed; (ii) no significant difference between the concentrations of *IL1B* in saliva between the case and control subjects and between the genotypes of these individuals and the concentrations of this cytokine. **Conclusions:** We conclude that, in the sample evaluated, the *IL1B* c. 3954C > T polymorphism did not present as an etiological factor for periodontitis.

Keywords: Interleukin-1 beta, periodontal disease, polymorphism

Introduction

Periodontitis is a complex infectious disease, which causes destruction of the teeth supporting tissues. In the clinical course of the disease it might be observed gingival inflammation with consequent periodontal pockets formation due to the periodontal ligament destruction, in more advanced cases, culminates in alveolar bone resorption which results in the tooth loss.[1,2]

Because of the multifactorial nature of the disease, several factors have been related to its prevalence and severity. Besides the composition and characteristics of the individual oral microbiota, which is essential for triggering mechanisms related to the disease, inadequate oral hygiene, age, smoking, as well as behavioral and demographic characteristics have been related to the periodontitis. Smoking, for instance, is considered as a major risk factor for its development, for in smokers the disease progresses faster and in a more severe way.[3,4] In women, the influence of ovarian hormones also seems to be a predisposing factor to the disease, which makes this gender more susceptible to periodontitis.[4,5] Nevertheless, studies also point to an important role of genetics in the onset of disease progression, related to expression and secretion of molecules of the immunoinflammatory response against periodontal pathogens that comprise the oral microbiota.[6-8]

The pathogenesis of periodontitis is the result, therefore, from the action of oral pathogens that trigger the immune response, which are present in various tissues of the oral cavity, attached through the biofilm. The biofilm is formed by bacterial micro-colonies and extracellular matrix composed by more complex organic compounds such as long-chain polysaccharides and simpler as proteins and lipids.[9-11] As a consequence of this heightened exposure to

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periodontopathogenic microbiota, the gingival epithelium cells activated by substances produced and/or secreted by these bacteria trigger a signaling cascade that results in activation of the primary defense cells such as monocytes and macrophages, stimulating them to produce and secrete pro-inflammatory cytokines, among which stands out the interleukin-1 β (*IL1B*).[12,13] Among activities performed by *IL1B* in the inflammatory process are included the abilities to stimulate bone resorption by osteoclasts, signaling inhibition of bone restructuring, prostaglandins production, stimulation of neutrophil degranulation, stimulus to increase leukocyte adhesion and production of metalloproteinases. These activities together are crucial to the destruction of tooth support tissues, typical in the development and progression of periodontitis.[13]

Among the genetic factors that may influence the immune response exacerbation, it is highlighted the single nucleotide polymorphisms (SNPs), which are genomic variants that may be related to some considerable phenotypic expression, depending on environmental influence. In periodontitis, as well as in other complex diseases, multiple genes, and variants, especially those which encode immune response related elements, may partially contribute to disease susceptibility or severity. Studies have reported associations between cytokine gene polymorphisms and periodontitis in distinct populations, and contradictory results have been found among different ethnic groups, even in the same country.[8,14] Among these variants, the polymorphism c. 3954C > T (SNP rs1143634) in the *IL1B* gene is the target of many studies.[14,15] In about 20 studies, mostly in Caucasian population samples, a positive association between periodontal disease and the *IL1B* c. 3954C > T has been observed.[8,14,16,17]

The present study aimed to evaluate the association of the polymorphism *IL1B* c. 3954C > T and periodontitis in a population sample from Vitória da Conquista, Bahia, Brazil.

Materials and Methods

Study population, anamnesis and clinical examination

The present study employed a case-control design. Sample study is characteristically multiethnic and composed of 347 subjects aged between 15 and 71 years (69 men and 278 women, 35 mean age). All patients included in this study were receiving dental care by the public health system in Vitória da Conquista, Bahia, North-East of Brazil. Edentulous individuals and those who made use of antimicrobial therapy in the 3 months prior to the clinical evaluation and sample collection were excluded from this study.

The study protocol was approved by the Ethics Committee of State University of Southwest Bahia under the protocol registration 071/2009 and written informed consent was obtained from all patients or the parents of participants under the age of 18.

The individuals were initially submitted to anamnesis and clinical examination performed by a dentist at the health public unit. Among the preestablished and evaluated diagnostic criteria for periodontitis are included: (i) Depth probe ≥5 mm, (ii) observation of gingival bleeding on probing, (iii) the occurrence of inflammation and dental mobility by destruction of the tooth supporting tissues.[18] Based on these criteria, patients were divided into two distinct groups: Case-composed of 134 patients with periodontitis- and control-with 213 individuals with periodontal health evidence.

Sample collection

For purposes of genomic DNA extraction, epithelial cells of patients were collected by scraping the oral mucosa using sterile swabs. After this procedure the swabs were placed in sterile 2 mL tubes, sealed, identified and stored at −20°C for later DNA extraction. Aiming to assess the concentration of *IL1B* we asked the patients to provide a volume of saliva ≥3 mL, which were packaged in 15 mL sterile tubes and stored at −20°C for subsequent dosing. Saliva was not stored longer than 2 months.

DNA extraction and genotyping

Genomic DNA was extracted by alkaline solution technique.[19] Then, DNA was transferred to sterile tubes and stored at − 20°C until genotyping.

Genotypes were determined by polymerase chain reaction (PCR) amplification followed by digestion with restriction endonuclease (PCR-restriction fragment length polymorphism). Concerning the amplification conditions it was used 20 ρmol of each primer and 1 U of *Taq* DNA polymerase. Concerning to the cycling conditions, the annealing temperature was standardized to 61.5°C. A 182 bp-fragment was amplified using primers described elsewhere.[20] Restriction was performed with *TaqI* endonuclease, and after digestion at 65°C for 4 h, the products were resolved in 3% agarose gels in 1X Tris/Borate/EDTA, stained with ethidium bromide (0.5 mg/mL). Gels were visualized on an ultraviolet transilluminator and photographed at appropriate photo documentation system.

Determination of interleukin-1 β levels

To determine the *IL1B* cytokine levels in saliva of patients, it was used the enzyme-linked immunosorbent assay (ELISA) technique according to the protocol and the instructions given by the manufacturer's kit used. The reactions were performed in ELISA plates containing 96 wells, which were subsequently read from the apparatus Vivid Vision Microplate Reader at a wavelength of 450 ηm. The concentrations were obtained by comparing the absorbance found by the concentration curve obtained by serial dilution according to the standard kit.

Statistical analysis

Demographic data, information from clinical examination and history as well as the genotypes for each individual were

plotted using Statistical Package for the Social Sciences (SPSS)® Statistical Software Inc., version 16 (Chicago, USA). Genotypes were determined by simple counting. Data obtained from clinical history analysis, clinical examination, genotyping and cytokine quantification were statistically evaluated. Other statistical calculations involved in this work include Fisher Exact test for parametric data and Mann–Whitney and Kruskal–Wallis tests for nonparametric data, with the first group performed by SPSS® version 16 software and the second in GraphPad Prism®, Graph Pad Software Inc., version 5 (San Diego, USA). The strength of the associations was determined using odds ratio (OR) calculations and 95% confidence intervals (CI).

It was adjusted the multivariate logistic regression model in order to identify potential confounding factors and to estimate odds ratios (OR) for periodontitis, controlling for the effects of other variables included in the final model. Were considered to enter the model the variables with a $P < 0.20$ in the bivariate analysis, remaining in the final model, variables with $P < 0.05$. A multivariate analysis was performed using Stata version 10.0 (Stata Corp., College Station, USA).

Results

The studied sample consisted of a total of 347 individuals of whom 59% were older than 30-year-old, 80% were female, and 77% had only completed primary school. The age group consisted of people up to 30 years was positively associated with periodontitis (OR = 2.54; confidence interval [CI] 95%: 1.88–4.85). The other analyzed factors such as smoking, gender, education, and the presence of systemic diseases was not statistically associated with the presence of periodontitis. The signs and symptoms assessed by clinical examination and history interview were positively associated with periodontitis showing the following variables: Tooth mobility (OR = 2.61, 95% CI: 1.58–4.33), gingival sensitivity (OR = 1.72, 95% CI: 1.00–2.97) and discomfort when chewing (OR = 1.64, 95% CI: 1.05–2.54).

Table 1 shows the OR to periodontitis with their respective CI 95% estimated by multivariate regression model. Risk factors were identified for periodontitis with OR ranging from 1.98 to 3.17 for the variables: Age ≥30 years, smoking and tooth mobility range.

The selected sample for the present study was also evaluated for c. 3954C > T *IL1B* gene polymorphism association with periodontitis. The found genotype distribution for the 347 evaluated subjects was 60.5% for the CC genotype, 22.2% for genotype CT and 17.3% for the TT genotype. The observed genotype frequencies did not differ significantly between case and control (p = 0.457) [Table 2].

In order to investigate the relationship between genotype frequency and periodontitis, individual genotypes were compared with each other in case and control groups [Table 3]. The CC genotype was showed as a possible protective factor for illness (OR = 0.77, 95% CI: 0.5 – 1.2) and CT genotypes (OR = 1.39 , 95% CI : 0.82 – 2.37) and TT (OR = 1.17 , 95% CI 0.65 - 2.12) as associated factors. However, no significant difference was found.

The dosage of local *IL1B* expression was performed in order to assess its relationship with the polymorphism *IL1B* c. 3954C > T and periodontitis [Figure 1]. The case and control groups did not differ regarding this cytokine concentrations, indicating no relationship between periodontitis and the inflammation status ($P = 0.7370$). The *IL1B* concentrations also showed no differences between the groups when they were stratified by genotypes for the polymorphism ($P = 0.6444$) [Figure 2].

Discussion

Periodontitis is characterized by the interaction of specific pathogens in the oral cavity and the immune responses of the organism. The disease has a complex character, and therefore, its etiology is intrinsically linked to different environmental factors such as age, lifestyle habits that include poor oral hygiene, and also the inheritance of a set of genes, which are associated to the organism inflammatory responses.[21]

Table 1: Factors associated with periodontitis in the population sample analyzed in Vitória da Conquista, Bahia-Brazil

Variables	Adjusted OR (CI 95%)	P*
Age		
≥30 years	3.17 (1.90; 5.28)	<0.001
Smoking		
Yes	2.19 (1.11; 4.32)	0.023
Tooth mobility		
Yes	1.98 (1.13; 3.47)	0.016

*Multivariate logistic regression model. CI: Confidence interval; OR: Odds ratio

Table 2: Allele and genotype frequencies of *IL1B* c. 3954 C>T observed in individuals with periodontitis and in periodontally healthy population

Genotypes	*n* (%)				*P*
	Periodontitis case		Periodontitis control		
	n=134		*n*=213		
CC	76 (56.7)		134 (62)		0.457
CT	34 (25.4)		43 (20.2)		
TT	24 (17.9)		36 (16.9)		
Allelic frequency (%)	*C*	*T*	*C*	*T*	0.306
	69.4%	30.6%	73.0%	27.0%	

IL1B: Interleukin-1β

Table 3. Genotypic profile of individuals in the case and control groups in relation to periodontitis

Variables	*n* (%)		Odd ratio	CI 95%	*P*
	Periodontitis case *N*=134	Periodontitis control *N*=213			
IL1B- Dominant model					
CC	76 (22)	134 (38)	1.00		
CT+TT	58 (17)	79 (23)	0.77	0.49;1.25	0.2612
IL1B- Codominant model					
CC	76 (56.7)	134 (62.9)	1.00		
CT	34 (25.4)	43 (20.2)	1.39	0.82;2.37	0.219
TT	24 (17.9)	36 (16.9)	1.17	0.65;2.12	0.5900
IL1B- Recessive model					
TT	24 (7)	36 (10)	1.07	0.61;1.90	0.8842
CC+CT	110 (32)	177 (51)	1.00		

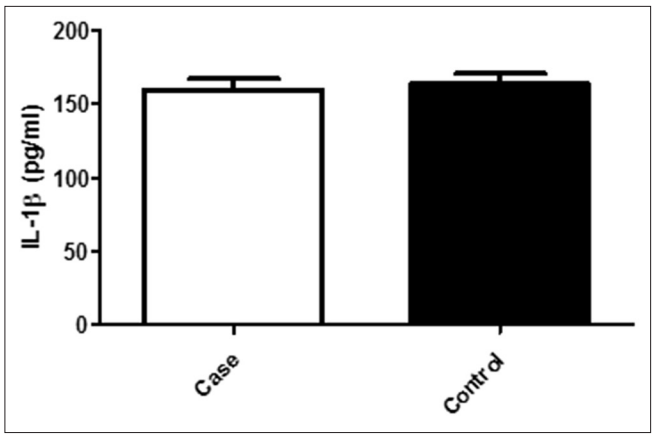

Figure 1: Quantification of interleukin-1 β cytokine in saliva of individuals in the case and control groups using enzyme-linked immunosorbent assay. Analysis by Mann–Whitney

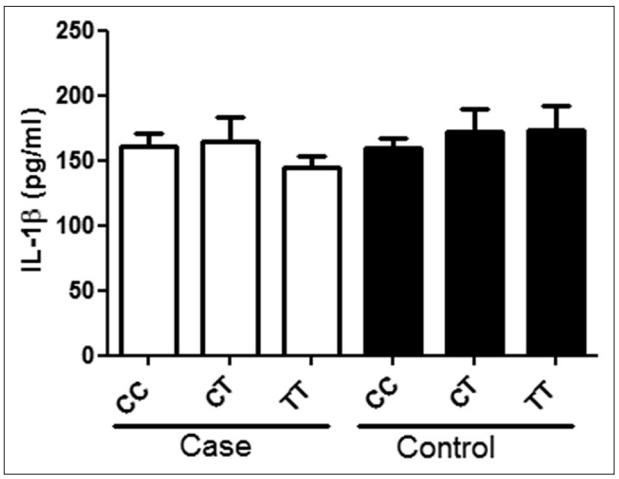

Figure 2: Quantification of interleukin-1 β (IL1B) cytokine in the saliva of individuals of the case and control groups separated by c.3954 C>T IL1B polymorphism genotype, using enzyme-linked immunosorbent assay. Analysis by Kruskal–Wallis

Regarding the demographic characteristics of individuals affected by periodontitis, the present study revealed that 74.63% of patients in this group are older than 30 years, 79.1% completed only elementary school and a higher percentage distribution to men (40.6%) than to women (38.1%). Similar results were observed in a study conducted with a North American population sample. In this study, it was reported that periodontitis affects mainly males, with low level of education and who are older than 30 years.[22]

It was observed in our study that although smokers were equally allocated in case and control groups, there is a relationship of risk for periodontitis in this population, confirming previous findings regarding smoking as a risk factor.[23] Smoking cause injury to the oral cavity and respiratory tract tissues also triggers various harmful oral/dental health responses that culminate in increased release of proinflammatory mediators and depletion of the immunological defense mechanisms. These two events associated with a higher propensity result in the establishment of periodontopathogenic microorganisms, and, therefore, smokers have been characterized in several studies as subjects more susceptible to the periodontitis emergence and severity.[24,25]

Coexistence with other metabolic diseases such as diabetes mellitus has also been described as a risk factor for periodontitis. In diabetes mellitus, the presence of hyperglycemia, metabolic alterations and/or oxidative stress, generate physiological changes that cause exacerbation of inflammatory responses due to increased cell activation and cytokine secretion, making diabetic patients more susceptible to periodontitis development with a tendency to present more severe clinical outcome.[26,27] In the present study population, seven subjects (2% of the total population) reported themselves as bearers of diabetes mellitus. With regard to the allocation in the case and control groups, four individuals, equivalent to 57.1% were in the case group, with OR = 2.154. Possibly no statistically significant difference was observed due to the small number of diabetics in the population.

With the exacerbation of inflammatory processes in response to the stimulus generated by pathogenic bacteria in the oral cavity, the progressive destruction of the supporting tissues, which manifests with characteristic signals: Sensitivity and gingival bleeding, increased tooth mobility and uncomfortable in chewing.[28,29] In this work, for tooth mobility, gingival sensitivity and discomfort when chewing, statistical differences between case and control groups were found. The gingival bleeding was presented as a risk factor for periodontitis (OR = 1.26), with no statistical difference between groups ($P = 0.311$). Once the bleeding gum is not considered as a pathognomonic clinical sign for periodontitis, it can be observed in several other diseases that affect the oral cavity, from the most complex to the simplest, such as gingivitis.[30] Considering that gingivitis is an inflammatory process analogous to periodontitis, but milder and reversible, it is possible that patients not diagnosed with periodontitis, might have presented gingival bleeding, so that this variable did not yield statistically significant differences between the evaluated groups.[30-33]

Besides the mentioned factors, for periodontitis are also recognized several genetic factors that could make an individual more susceptible to the development of this disease and/or its severity. Among these we highlight genes and allelic variants, especially those encoding proteins that play important roles in the host immune response. Particularly, there is the *IL1B* gene polymorphism c. 3954C > T, which has been the subject of studies seeking to relate it to an increase in the periodontitis incidence and/or severity. Nevertheless, the results have not been shown fully coincident findings in different populations.[34-37]

Several studies conducted that sought to observe a genotype/phenotype relationship for *IL1B* c. 3954C > T polymorphism and periodontitis, showed statistically significant differences in genotype frequencies between case and control groups.[38-40] In the present study, it was not possible to establish a relationship between the polymorphism and periodontitis in the analyzed sample, although we observed a higher frequency for the CC genotype among control subjects compared to patients with periodontitis, suggesting a protective factor to the disease. Masamatti *et al*.[38] conducted a study to assess the genetic *IL1B* polymorphism c. 3954C > T influence in periodontitis in an Indian population sample and observed statistically significant differences when comparing the genotype frequencies between subjects with chronic and aggressive periodontitis and healthy individuals for these polymorphism. The authors concluded that the *IL1B* c. 3954C > T polymorphism was associated with chronic periodontitis in Indian population of Karnataka ethnicity.

In another study, involving a population sample from the Yemen, Al-hebshi *et al*.[39] observed a *T* allele frequency of 47.5% among patients with periodontal disease and 40% in clinically healthy subjects. These findings are also divergent to those obtained in the present study since the authors reported the existence of a statistically significant association between the polymorphism and periodontitis.

A study conducted in a Brazilian Southeast population sample, also pointed to a positive association between the *IL1B* c. 3954C > T polymorphism and periodontitis. The studied population showed a frequency of 26.5% for the *T* allele among the 117 individuals in the case group and 20% among the 175 control individuals.[40] Our data regarding the allele and genotype frequencies resemble those observed by the authors of this study. Nevertheless, in our sample, the analysis of the relationship between the polymorphism and periodontitis showed no statistical significance.

Similarly to our results, Bascones-Martínez *et al*.,[41] analyzing a population sample from Spain, have not shown an association between *IL1B* c. 3954C > T and the periodontitis. In this study, the control group revealed the following genotype distribution: About 48% were carrying the CC genotype, 48% CT and 4% TT. Among the clinically ill patients, 64% had the CC genotype, 32% CT and 4% the TT genotype. No significant difference was observed in genotype distribution when compared case and control groups. The evaluation of another Brazilian population sample, also have not identified such association with periodontal disease. The frequency of the *T* allele was estimated at 78.5% and 62.5% among patients with moderate and severe periodontitis, respectively, and 25% among the control group.[42] For the polymorphism analysis in a Japanese population sample, the observed frequency for the *T* allele was 2.6 and 2.8% for groups of individuals with and without periodontitis, respectively.[43]

The c. 3954C > T *IL1B* polymorphism may also be related to the increased activity and/or concentration of *IL1B*, and in some cases, its low concentration might be associated with less severe periodontitis.[44] The levels of certain inflammatory cytokines in saliva of patients with periodontitis have been shown to be elevated. However, in the present study, *IL1B* levels in the patient's saliva, showed no significant difference between case and control groups or between the CC, CT and TT genotypes of each of the groups. A work conducted in Turkey evaluated the association between the prevalence, severity of periodontitis and *IL1B* c. 3954C > T with cytokine salivary concentrations and as a result it was observed that the polymorphism studied is not necessarily related to the increased *IL1B* concentration in saliva, confirming the findings of the present work.[45]

Conclusions

Given the multifactorial nature of the disease under study, several factors may justify the noncompliance of a statistically significant association between the

polymorphism and periodontitis. Among these, are: (i) Other genes-not analyzed in this study, which may have influence on this relationship; (ii) the role played by environmental factors in the disease outcome that can overcome the genotype factor would include here, for instance, poor oral hygiene.

Despite the c. 3954C > T polymorphism have not been shown as itself as a marker of risk for periodontitis in the present study population, further investigation on the genetic variations and disease in question are necessary to elucidate the determinants genetic factors in the onset and progression of periodontitis, which will enable the development of better strategies for disease diagnosis.

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