

Research

*Corresponding author

Sydney Correia Leão, MD

Medical Pathology Assistant

Department of Pathology

Federal University of São Paulo

São Paulo, SP, Brazil

E-mail: sydneyleao@hotmail.com

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Polymorphic Genetic Markers in Patients with Chronic Periodontitis

Sydney Correia Leão, MD^{1*}; Débora Machado Barreto, BSc, MSc²; Ricardo Vieira da Costa, BSc, MSc²; Rosilene Calazans Soares, MD, MSc, PhD²; Tânia Maria de Andrade Rodrigues, MD, MSc, PhD²; Marcus Vinicius de Aragão Batista, BSc, MSc, PhD³; Margareth Aparecida Meneses de Almeida, OD, PhD⁴

¹Department of Pathology, Federal University of São Paulo, São Paulo, SP, Brazil

²Department of Morphology, Federal University of Sergipe, São Cristóvão, SE, Brazil

³Department of Biology, Federal University of Sergipe, São Cristóvão, SE, Brazil

⁴Department of Odontology, Federal University of Sergipe, Aracaju, SE, Brazil

ABSTRACT

Introduction: Chronic periodontitis (CP) is an inflammatory disease caused by the interaction among dental plaque, periodontal tissues, and host immune responses, which causes bone resorption. Interleukin-6 (IL-6) and matrix metalloproteinase-1 (MMP-1) plays an important role in the periodontium destruction. Some authors have studied polymorphisms in these genes but the results were controversial.

Objective: The aim of this study was to evaluate the association of polymorphisms in the promoter region of genes IL-6 (-174) and MMP-1 (-1607) with CP in a Northeastern Brazilian population.

Methods: A case-control study of 64 cases of CP patients and 25 healthy subjects was performed. Genomic DNA was isolated from buccal mucosa and two single nucleotide polymorphism in IL-6 (-174 G/C) and MMP-1 (-1607 1G/2G) genes were analyzed using polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR-RFLP).

Results: Regarding to results, there were no statistically significant differences between patients with CP and control group, nor to genotypes ($p=0.734$) nor to alleles ($p=0.763$), with respect to IL-6 polymorphism. For MMP-1 polymorphism, also we found no statistically significant difference in genotype ($p=0.607$) and allele ($p=0.237$) distribution between individuals with CP and the control group.

Conclusion: We concluded that the polymorphism of the studied genes were not associated with susceptibility to CP in this Northeastern Brazilian population.

INTRODUCTION

Periodontitis is a chronic inflammatory disease caused by the interaction among biofilm, periodontal tissue, and host vascular and cellular responses, which leads to bone tissue resorption.¹ Chronic periodontitis (CP) prevalence ranges from 40% to 80% in general population, which is due to regional differences and methodological variations in the studies.² CP affects the periodontium, and although bacteria are essential for the disease initiation, bacteria number and type are not sufficient to explain the differences in their severity.³

Besides the pathogenesis of chronic lesions is associated with the interaction among biofilm, host response and influences of secondary factors, genetic polymorphism may be determinant of certain immunological aspects of the disease.⁴

Smoking is an important epidemiological factor associated with CP⁵ as well as some systemic conditions such as diabetes and cardiovascular disease, especially hypertension. Periodontal disease is considered the 6th complication of diabetes.⁶ A longitudinal study demonstrates an increased risk of 50% to 100% of having oral conditions prior to the occurrence of cardiovascular diseases.⁷

Interleukin-6 (IL-6) is a multifunctional cytokine with a central role in host defense.⁸ However, it also stimulates osteoclast activity, making it a potent inducer of alveolar bone resorption.⁹ Matrix metalloproteinases (MMPs) are families of metal dependent proteolytic enzymes, which are capable of degrade all components of the extracellular matrix, including various types of collagens and basement membrane components. MMPs also actively participate in bone resorption that occurs in periodontitis.¹⁰ Polymorphism in several genes, such as IL-6 and MMP-1, have been associated with the pathogenesis of the disease. Genetic polymorphisms can be defined as the exchange of a nucleotide (adenine - A, guanine - G, thymine - T and cytosine - C) for another at a given site of a gene (locus) in general population whose frequency is greater than 1%.¹¹ Currently, research has been conducted in order to define the profile of these genetic markers and their association with CP, but the results are still controversial.¹²

The aim of this study was to investigate the possible association among IL-6 (-174) and MMP-1 (-1607) polymorphisms and the susceptibility to CP in a population from North-eastern Brazil.

METHODOLOGY

This is a quantitative, field, observational and cross-sectional study, comprising patients with chronic periodontitis and control group. They were randomly selected at the Department of Dentistry of the Federal University of Sergipe, on the city of Aracaju, Brazil. The study was approved by the Ethics Committee of the Federal University of Sergipe (CEP/UFS) (CAAE-0225.0.107.000-11) and all participants signed an informed consent form.

Patients with CP were diagnosed by dentistry professors of the Federal University of Sergipe, according to the protocol of the American Association of Periodontology (1999). Clinical diagnosis of periodontitis was based upon the measurement of the clinical attachment level (CAL).¹³ The subjects were divided into two groups: healthy (individuals who had no change in the periodontium); and chronic periodontitis (individuals who had $CAL \geq 3$ mm).

Individuals who reported chronic use of anti-inflammatory drugs, HIV infection, or any other serious illness that could cause impairment of the immune system, as well as those who needed immunosuppressive chemotherapy were excluded from the study.

Genomic DNA was isolated from buccal mucosa cells collected by scraping with a sterile swab. Genomic DNA extraction of buccal swab was carried out using the AxyPrep Multi source Genomic DNA Miniprep kit (Axygen Biosciences, Andover, USA), according to the manufacturer. The DNA was stored at -20 °C until PCR analysis.

IL-6 (-174) and MMP-1 (-1607) polymorphisms were assessed by PCR amplification and digestion as previously described.^{11,14} The reactions were performed in the Veriti® 60-Well Thermal Cycler (Life Technologies, Sao Paulo, Brazil).

Restriction digestion products were separated in 3% agarose electrophoresis gel stained with Blue Green Dye I® (LGCBIO™, Sao Paulo, Brazil), and visualized on an ultraviolet transilluminator (Spectroline, Westbury, New York, USA).

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical tables involving all variables of the study were generated, which formed the basis for the analysis. We performed the nonparametric chi-square test, to check the possibility of association between variables, using a significance level of 5 %. In addition, a multiple logistic regression test was carried out using BioEstat 5.0.

RESULTS

This study included 89 individuals, 25 subjects without periodontal change (control group) and 64 individuals with CP (Table 1). The samples had similar DNA concentrations, ranging from 22.1 ng/μl (with a purity of 1.95) to 27.5 ng/μl (with a purity of 1.84).

	Chronic Periodontitis N(%)	Control N(%)
Total sample: 89	N=64(100%)	N=25(100%)
Gender:		
Male (%)	18(28.1%)	6(24.0%)
Female (%)	46(71.8%)	19(76.0%)
Age (years)	20-68	14-48
Mean and standard deviation (*)	48.2±1.5	30.9±1.9
Smoking:		
Smoking (%)	10(15.6%)	2(8.0%)
Non-smoking (%)	54(84.4%)	23(92.0%)
Systemic diseases:		
anemia	13(20.3%)	2(8.0%)
hypertension	16(25.0%)	0(0%)
diabetes	3(4.7%)	0(0%)
anemia and diabetes	1(1.6%)	0(0%)
diabetes and hypertension	4(6.3%)	0(0%)
Hepatic disease	1(1.6%)	0(0%)
No disease	26(40.6%)	23(92%)

Table 1: Clinical characteristics of the studied subjects.

We have found no statistically significant difference between patients with CP and the control group, nor with respect to IL-6 genotypes (-174) ($p=0.734$) neither to the alleles ($p=0.763$) (Table 2). In addition, we have found no statistically significant difference with respect to IL-6 genotypes (-174) among smoking ($p=0.638$) and non-smoking ($p=0.229$) individuals with CP and control group (Table 2).

IL-6(-174)	Chronic Periodontitis N(%) N=64(100%)	Control N(%) N=25(100%)	p(*)
Genotypes:			
G/C	29(45.3 %)	10(40.0%)	0.734
G/G	34(53.1%)	14(56.0%)	
C/C	1(1.6%)	1(4.0%)	
Alleles:			
G	97(62.2%)	38(64.4%)	0.763
C	59(37.8%)	21(35.6%)	
MMP-1(-1607)	Chronic Periodontitis N(%) N=64	Control N(%) N=25	p(*)
Genotypes:			
1G/2G	4(6.3 %)	1(4.0 %)	0.607
2G/2G	58(90.6 %)	24(96.0 %)	
1G/1G	2(3.1 %)	0(0.0 %)	
Alleles:			
1G	8(6.3 %)	1(2.0 %)	0.237
2G	118(93.7 %)	49(98 %)	

Table 2: Distribution of genotypes and alleles of IL-6 (-174) and MMP-1(-1607) gene polymorphisms between subjects with chronic periodontitis and control group.

With respect to patients with CP who also presented some systemic diseases as diabetes, anemia, hypertension and hepatitis, we observed that the G/G genotype was found in nine subjects who had anemia while four of them presented G/C genotype. With regard to hypertension, the results were similar between genotypes, eight individuals with G/G genotype and eight with G/C genotype. Two patients with diabetes presented the C/G genotype while the G/G genotype was observed in one patient. None of the patients with CP and systemic disease had the C/C genotype. Among patients with CP and no systemic diseases, 12 subjects had the G/G genotype, 13 had the G/C genotype and only one had the C/C genotype. In total, we did not find statistically significant difference ($p=0.61$). The G/G genotype of IL-6 gene polymorphism (-174) was represented by 229 bp and 173 bp bands, and the C/G genotype was represented by 229 bp, 173 bp and 122 bp bands.

No statistically significant difference was found in the genotype ($p=0.607$) and allele ($p=0.237$) distribution of MMP-1 gene polymorphism (-1607) between individuals with CP and the control group (Table 3). We have found no statistically significant difference with respect to MMP-1 genotypes (-1607) between patients with CP and the control group among smoking ($p=0.640$) and non-smoking ($p=0.617$) individuals (Table 3).

In addition, we have compared patients with CP and systemic diseases to the control group. We observed that nine patients with CP and anemia had the 2G genotype, two presented the 1G/2G genotype, and two showed the 1G genotype. With regard to hypertension, we observed the G/G genotype in 16 patients. Among patients who had diabetes, only the 2G genotype was found (three patients). No patients with CP and diabetes and hypertension presented the 1G and 1G/2G genotypes. Twenty-

four patients with CP without systemic disease presented the 2G/2G and 1G/2G genotypes but no C/C genotype. In total, we have found no statistically significant difference ($p=0.32$).

The multivariable logistic regression test showed that the studied genetic markers did not interfere in CP. We also observed that smoking individuals is six times more likely to develop periodontitis. The 1G/2G genotype of MMP-1 gene (-1607) was represented by 118 bp, 89 bp and 29 bp bands, and the 1G/1G genotype was represented by an 89 bp size band.

DISCUSSION

In the present study, we have examined the contribution of two polymorphisms (in IL-6 and MMP-1 genes) for the susceptibility to chronic periodontitis in a Northeastern Brazilian population. Although several authors have investigated the association between polymorphisms and CP, still there are controversial results, which demonstrate the relevance of this study. In our study, the G/G genotype of IL-6 gene was observed in 53.1% and 56% of patients with CP and healthy subjects, respectively. This result suggests that, in our population, the G/G genotype are not associated with CP, which disagrees with other studies.^{12,14-16}

In one study conducted in India,¹⁵ patients with CP had a higher frequency of G/G genotype (66.67%) than healthy patients (13.33%). Thus, they suggested that the G/G genotype may have an important role in the progression of CP. However, in this study, the lack of association could be a consequence of the small sample size. In Germany,¹⁷ the authors observed a significant difference between the control group and CP patients. The C/C genotype of IL-6 gene (-174) was found in 27.6% of the control group and in 41.9% of the CP group.¹⁷ We believe

IL-6 (-174)	Chronic Periodontitis N(%)	Control N(%)	p(*)
Smoking:			
GC	2(20.0 %)	1(50.0 %)	0.638
GG	7(70.0 %)	1(50.0 %)	
CC	1(10.0 %)	0(0 %)	
Total	10(100 %)	2(100 %)	
Non-smoking:			
GC	27(51.0 %)	9(39.0 %)	0.229
GG	26(49.0 %)	13(56.0 %)	
CC	0(0 %)	1(4.3 %)	
Total	54(100 %)	23(100 %)	
MMP-1 (-1607)			
	Chronic Periodontitis N(%)	Controle N(%)	p(*)
Smoking:			
1G/2G	1(10 %)	0(0 %)	0.640
2G/2G	9(90 %)	2(100 %)	
Non smoking:			
1G/2G	39(5.6 %)	1(4.3 %)	0.617
2G/2G	48(90.5 %)	22(95.6 %)	
1G/1G	2(3.8 %)	0(0 %)	

Table 3: Distribution of genotypes of IL-6 (-174) and MMP-1 (-1607) genepolymorphisms in smoking and non-smoking individuals.

that this genetic profile could be specific to European populations. This could be an explanation to the differences observed in all studies carried out in Brazil and of the results of the study conducted in India.¹⁵

In Bahia, Brazil, differences in genotypes of IL-6 polymorphism in patients with CP and healthy patients was not statistically significant ($p=0.126$). However, the frequency of the G allele was higher in patients with CP than in the healthy group, and this difference was statistically significant ($p=0.03$).¹⁸ The results of our study did not present this genetic pattern (G allele frequency of 64.4% in the control group and 62.2% in the group with CP), and this difference was not significant ($p=0.763$). We suggest that further studies should be conducted with a large sample size in Northeastern Brazil in order to confirm this association.

In our study, among the smoking individuals with CP, there was three times more G/G genotype (70%) than C/G genotype (20%), while the control group presented an equal distribution. In addition, in CP patients, there was a correlation between the frequency of IL-6 G/G genotype and age (Pearson $r=-0.3807$), and between G/G genotype and smoking (Pearson $r=-0.2998$). These results point to the evidence that the frequency of G/G genotype is relatively higher in young smoking individuals.

Although the differences were not statistically significant in both groups, smoking ($p=0.638$) and non-smoking ($p=0.229$), we believe that in this population, the G/G genotype might indicate a tendency of increasing susceptibility to CP in

young patients who smoke. However, the small number of samples should be a limiting factor in order to establish this association. Smoking was included in a study related to polymorphisms in IL-6 gene.¹⁴ Furthermore, the authors found no significant difference between smoking and non-smoking individuals in the distribution of the allele polymorphism in IL-6 gene.

The frequency of the 2G/2G genotype in the group with CP (90.6%) and the control group (96%) was similar. Additionally, there was no significant association between the MMP-1 (-1607) polymorphism and susceptibility to CP ($p=0.607$). Our results are in agreement with some other studies,^{12,19,20} but they are in disagreement with others.^{21,22} These discrepancies may reflect the involvement of variable risk alleles in different populations with diverse influencing factors such as genetic admixture, regional differences in gene frequencies, gene-environment interactions, and a poor sample selection of extreme phenotypes. In fact, we observed that most studies considering extreme phenotypes (especially severe diseases) were able to detect an association between the MMP-1 (-1607) gene polymorphism and CP. In this way, these data might indicate that the MMP-1 (-1607) gene polymorphism could be associated with CP severity rather than susceptibility.¹²

In this study, with respect to the MMP-1 polymorphism, there was no significant difference between the smoking ($p=0.640$) and non-smoking ($p=0.617$) groups, which was consistent with of another study.²³ consequently, we suggest that there is no association between MMP-1 (-1607) polymorphism and susceptibility to CP in smoking patients.

An *in vitro* study²⁴ concluded that the MMP-1 (-1607) polymorphism has functional significance in controlling the level of transcription and protein production of MMP-1 in periodontal ligament cells, which implies that subjects with CP with the 2G allele may have severe degradation of the extracellular matrix. This may explain the high number 2G allele in patients with advanced periodontitis in some studies.²⁰⁻²² However, the lack of association between MMP-1 and CP suggests that the increase in mRNA transcription might not necessarily lead to the increase in enzyme activity on the extracellular matrix, as confirmed in other populations.^{19,23,25}

CONCLUSION

In conclusion, our results suggest that there is no association between IL-6 (-174) and MMP-1 (-1607) gene polymorphisms and the susceptibility to CP. This lack of correlation was also observed taking into account several risk factors such as smoking and the presence of systemic diseases. However, the small sample size could be a limiting factor. Therefore, further studies should be conducted with a larger sample size in order to confirm if there is an association between these polymorphisms and CP. This knowledge is extremely relevant because it may serve as the basis for the development of diagnostic, preventive and therapeutic strategies that could improve the clinical treatment of chronic periodontitis.

CONFLICTS OF INTEREST: None.

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