UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA

JÔICE DIAS CORRÊA

# ASSOCIAÇÃO ENTRE POLIMORFISMOS GENÉTICOS, CONDIÇÃO PERIODONTAL E NÍVEIS DE MEDIADORES INFLAMATÓRIOS NA PERIODONTITE CRÔNICA

Belo Horizonte
2013

Jôice Dias Corrêa

# ASSOCIAÇÃO ENTRE POLIMORFISMOS GENÉTICOS, CONDIÇÃO PERIODONTAL E NÍVEIS DE MEDIADORES INFLAMATÓRIOS NA PERIODONTITE CRÔNICA

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Faculdade de Odontologia da Universidade Federal de Minas Gerais como requisito parcial para a obtenção do grau de Mestre em Odontologia.

Área de concentração: Estomatologia
Orientadora: Profa. Dra. Tarcília Aparecida Silva
Co-orientador: Dr. Celso Martins Queiroz-Junior

Belo Horizonte
2013

C824a Corrêa, Jóice Dias.
2013 Associação entre polimorfismos genéticos, condição periodontal
T e níveis de mediadores inflamatórios na periodontite crônica/ Jóice Dias Côrrea, 2013.
76 fls.: il.
Orientadora: Tarcília Aparecida Silva
Co-orientador: Celso Martins Queiroz-Junior
Dissertação (Mestrado) - Universidade Federal de Minas Gerais, Faculdade de Odontologia.
1. Doenças periodontais - Teses. 2. Polimorfismo genético - Teses.
3. Mediadores da inflamação - Teses. I. Silva, Tarcília Aparecida.
II. Queiroz-Junior, Celso Martins. III. Universidade Federal de Minas Gerais. Faculdade de Odontologia. IV.Título.

BLACK D047

Elaborada pela Biblioteca da Faculdade de Odontologia da UFMG

**UNIVERSIDADE FEDERAL DE MINAS GERAIS**
**FACULDADE DE DONTOLOGIA**
**Programa de Pós-Graduação em Odontologia**

Dissertação intitulada **"Associação entre polimorfismos genéticos, condição periodontal e níveis de mediadores inflamatórios na periodontite crônica"**, área de concentração em **Estomatologia**, apresentada por **Jôice Dias Corrêa**, para obtenção do grau de **Mestre em Odontologia**, **APROVADA** pela Comissão Examinadora constituída pelos seguintes professores:

Dra. Tarcília Aparecida da Silva
Orientadora
FO-UFMG

Dr. Celso Martins Queiroz Júnior
Co-Orientador
FO-UFMG

Dr. José Eustáquio da Costa
FO-UFMG

Dra. Paula Rocha Moreira
ICB-UFMG

Prof. Dr. Saul Martins de Paiva
Coordenador do Colegiado do
Programa de Pós-Graduação em Odontologia

Belo Horizonte, 25 de janeiro de 2013.

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia
Av Pres.Antônio Carlos, 6627 - Pampulha
Belo Horizonte – MG - 31.270-901
Tel: (31) 3409 2470 Fax: (31) 3409 2472
Email: posgrad@odonto.ufmg.br

Ata da Comissão Examinadora para julgamento da Dissertação de Mestrado em Odontologia, área de concentração em **Estomatologia**, da candidata **Jôice Dias Corrêa**.

Aos 25 de janeiro de 2013, às 14:00 h, na sala de Pós-Graduação (3403) da Faculdade de Odontologia, reuniu-se a Comissão Examinadora, composta pelos professores Dra. Tarcília Aparecida da Silva, Dr. Celso Martins Queiroz Júnior, Dr. José Eustáquio da Costa e Dra. Paula Rocha Moreira. A Professora Dra. Tarcília Aparecida da Silva, Orientadora da Dissertação, na qualidade de Presidente da sessão, apresentou a Comissão Examinadora e declarou abertos os trabalhos. À candidata foi dado o tempo de até 50 (cinquenta) minutos para fazer a exposição oral sobre o seu trabalho **"Associação entre polimorfismos genéticos, condição periodontal e níveis de mediadores inflamatórios na periodontite crônica"**. Encerrada a exposição, foi iniciada a arguição, dentro do limite de tempo de 30 (trinta) minutos, pelos Professores Dra. Paula Rocha Moreira, Dr. José Eustáquio da Costa, Dr. Celso Martins Queiroz Júnior e Dra. Tarcília Aparecida da Silva, com limite de 30 (trinta) minutos para a resposta. Terminadas as arguições, A Presidente suspendeu os trabalhos por 10 minutos para que os examinadores pudessem decidir pelo resultado a ser dado à candidata. A Comissão Examinadora opta pela APROVAÇÃO da candidata. Para constar, lavrou-se a presente ata, que vai assinada por mim, Dra. Tarcília Aparecida da Silva, Presidente e pelos demais membros desta comissão examinadora. Belo Horizonte, 25 de janeiro de 2013.

Dra. Tarcília Aparecida da Silva
Orientadora
FO-UFMG

Dr. Celso Martins Queiroz Júnior
Co-Orientador
FO-UFMG

Dr. José Eustáquio da Costa
FO-UFMG

Dra. Paula Rocha Moreira
ICB-UFMG

## AGRADECIMENTOS

A Deus, por me guiar sempre e me dar as forças necessárias para superar todos os obstáculos.

Aos meus pais, por me moldarem nos preceitos da ética, com muito amor e mesmo não estando aqui continuam sendo meus exemplos de determinação e caráter.

À minha família por toda a atenção e carinho, especialmente aos meus irmãos Guilherme e Nilo por acreditarem e confiarem em mim e pelo apoio quando eu mais precisei.

À Carol, pelo incentivo, por acreditar em mim quando eu mesma duvidava e pela paciência e por todos os momentos que me ouviu falar sobre coisas que ela acha de outro mundo!

À Iara por sempre me colocar no rumo certo, à Lívia por me emprestar seu ombro em tantos momentos e à Virgínia por me fazer rir quando tinha crises de stress!

À minha orientadora, Profa. Tarcília Aparecida Silva, por me guiar nos caminhos da ciência, pela paciência com os meus erros, pela sabedoria compartilhada e por ter acreditado no meu potencial e investido em mim logo que terminei a graduação.

Ao meu querido co-orientador Celso, por ser um grande exemplo de determinação e trabalho, e por me ensinar tanto nessa caminhada mesmo antes de ser meu co-orientador e ainda ser um ótimo amigo.

À Mila, pelos ensinamentos no laboratório, compartilhando o início desse trajeto, pelos conselhos e pelas conversas.

Aos amigos e companheiros do Dental Club, Janine, Adriana, Soraia, Davidson, Izabella e Letícia, pelos momentos de ajuda científica, profissional, pessoal e pelos momentos de descontração.

Aos meus queridos amigos Glayce e Guilherme, por fazerem minha vida mais doce e celebrarem comigo cada novo passo nessa longa jornada.

Aos colegas do mestrado e Doutorado em Odontologia, por compartilharem seus conhecimentos, pela companhia agradável na IADR e nos encontros fora da UFMG.

Aos colegas do laboratório de Interação Microrganismo-Hospedeiro, do ICB, pela ajuda em tantos experimentos.

Aos professores Mauro Teixeira, Antônio Teixeira, Daniele Souza, José Estáquio e Ricardo Gomez, pelo apoio técnico, científico e orientações sempre valiosas.

À Inês Ferreira, pelo apoio técnico imprescindível.

À Renata Resende, por me ajudar tanto e com tanta disposição e paciência durante meu trabalho.

Aos pacientes e familiares que gentilmente colaboraram para a realização deste estudo, sem os quais ele não poderia existir.

“A imaginação é mais importante que o conhecimento.”

**Albert Einstein**

## RESUMO

A doença periodontal (DP) resulta da interação entre os mecanismos de defesa do hospedeiro com os microrganismos do biofilme dental. Essa interação é regulada por citocinas, que podem desempenhar papel protetor e/ou destrutivo na progressão da doença. Citocinas como IL-17 e TNF-α podem ser consideradas como pró-inflamatórias, enquanto outras, como IL-10, têm função na regulação da resposta imune. Os níveis destas moléculas e, consequentemente, o curso da DP podem ser modificados por polimorfismos genéticos. O objetivo deste trabalho foi avaliar a presença de polimorfismos nos genes *IL-17A, IL-17F, IL-10* e *TNFA* em pacientes com periodontite crônica e correlacioná-los com parâmetros clínicos da DP e com níveis dessas moléculas no periodonto e no soro. Observou-se que os alelos A dos genes *IL-17A (197)* e *IL-10 (1087)* associaram-se a um maior risco para DP. Além disto, o alelo A do gene *IL-17A* foi associado a parâmetros clínicos desfavoráveis, maior atividade de mieloperoxidase (MPO) e expressão aumentada de IL-17A e CXCL8/IL-8 nos tecidos periodontais. O genótipo GG de *IL-10* foi relacionado a níveis aumentados de IL-10 e menor expressão de CXCL10 nos pacientes com DP. Em contrapartida, o genótipo AG esteve associado a um maior número de sítios com sangramento à sondagem e maior atividade de MPO. Não foram observadas diferenças com relação aos polimorfismos dos genes *IL-17F* e *TNFA* e risco para DP. Estes resultados indicam que os alelos *IL-17A-197A* e *IL-10-1087A* estão associados com risco aumentado para a DP, provavelmente porque se associam a um padrão inflamatório exacerbado nos tecidos periodontais.



Palavras-chave: Doença periodontal, polimorfismo genético, mediadores inflamatórios.

Association between genetic polymorphisms, periodontal condition and inflammatory mediators levels in chronic periodontitis


## ABSTRACT

Periodontal disease (PD) results from the interaction of host defense mechanisms with the microorganisms of the oral biofilm. This interaction is regulated by cytokines that can play a protective and/or destructive role in disease progression. Some cytokines, such as IL-17, IFN-γ and TNF-α, promote inflammation. Others, such as IL-10, present a negative role in regulating the inflammatory response. The levels of these molecules and, consequently, the course of PD can be changed by genetic polymorphisms. The aim of this study was to evaluate the presence of genetic polymorphisms of *IL-17A*, *IL-17F*, *IL-10* and *TNFA* in patients with chronic PD and correlate the genotypes with clinical parameters of PD and cytokine levels in periodontal tissues and serum of patients. Individuals with the allele A of *IL-17 (197)* and *IL-10 (1087)* showed increased risk for PD, whereas no differences have been detected for *TNFA* and *IL-17F* polymorphisms. Furthermore, the allele A of *IL-17A* was associated with worse clinical parameters, higher activity of MPO and increased expression of inflammatory mediators (IL-17A and CXCL8/IL-8). The GG genotype of *IL-10* was correlated with increased levels of IL-10 and reduced expression of CXCL10 in periodontal tissues. On the other hand, the AG genotype carriers exhibited more sites with BOP and increased MPO activity. These results indicate that the *IL-17A-197A* allele and the *IL-10-1087A* allele are associated with increased risk for PD, probably because these genotypes correlate with a pattern of exacerbated inflammatory status in periodontal tissues.



Keywords: periodontal disease, genetic polymorphism, inflammatory mediators.

**SUMÁRIO**

## LISTA DE ABREVIATURAS E SIGLAS

CXCL: ligante de quimiocina CXC

DNA: ácido desoxirribonucleico

DP: doença periodontal

IL-: interleucina

*IL-17A*: gene da citocina IL-17A

*IL-17F*: gene da citocina IL-17F

*IL-10*: gene da citocina IL-10

IFN: interferon

MMP: metaloproteinase

MPO: mieloperoxidase

PG: prostaglandina

RANKL: ligante do receptor ativador do fator nuclear kappa-B

SNP: polimorfismo de nucleotídeo único

TNF: fator de necrose tumoral

*TNFA*: gene da citocina TNF-α

# 1 SÍNTESE BIBLIOGRÁFICA

## 1.1 DOENÇA PERIODONTAL

Doenças periodontais (DPs) são infecções associadas à destruição das estruturas de sustentação dos dentes. Ocorrem como resultado da interação entre o sistema imune do hospedeiro em resposta ao acúmulo de placa bacteriana (PAGE *et al*., 1997). Essa interação leva à inflamação dos tecidos periodontais, seguida de reabsorção do osso alveolar e consequente perda de inserção do ligamento periodontal (KINANE, BERGLUNDH, LINDHE, 2005). Este processo patológico apresenta diferenças na extensão e gravidade em um mesmo indivíduo e entre indivíduos, sendo as razões para isso multifatoriais (KINANE e LINDHE, 2005).

De acordo com Armitage (1999), duas categorias principais de DP foram estabelecidas: a periodontite crônica e a periodontite agressiva. A periodontite crônica apresenta uma taxa de progressão frequentemente baixa, sendo caracterizada por eventuais episódios de atividade da doença e por presença de placa ou cálculo compatíveis com a gravidade da doença (ARMITAGE, 1999). Por outro lado, a periodontite agressiva difere da periodontite crônica pela alta taxa de progressão, que leva a uma avançada destruição periodontal associada à perda precoce do dente. A quantidade de deposição microbiana é geralmente incompatível com a gravidade da destruição periodontal, e pode-se observar uma tendência familiar no aparecimento da doença (ARMITAGE, 1999).

A periodontite crônica afeta cerca de 30% da população mundial adulta. Dentre estes, cerca de 7-13% dos indivíduos desenvolverá formas de periodontite mais destrutivas (NARES, 2003). No Brasil cerca de 30% da população adulta do país apresenta bolsas periodontais em um ou mais sítios (BRASIL, 2011).

Além de causar a destruição dos tecidos de suporte dos dentes, a DP também pode afetar a saúde sistêmica do indivíduo. Vários estudos correlacionam a inflamação periodontal com alterações endócrinas, cardíacas, desordens autoimunes e na alteração do curso gestacional (MERCADO *et al*., 2001; MADIANOS *et al*., 2002; ALMAN *et al*., 2011, COSTA *et al*., 2012; QUEIROZ-JUNIOR *et al*., 2012).

## 1.2 PATOGÊNESE DA DP

Bactérias Gram-negativo anaeróbias estritas e anaeróbias facultativas, como *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia e Treponema denticola*, são os principais microrganismos associados com a causa e desenvolvimento das gengivites e periodontites (PEREA, 2004). De fato, é bem reconhecido que muitos indivíduos são portadores desses microrganismos sem manifestar DP. Nesse contexto, fica claro que, na maior parte da população, o biofilme presente no meio oral está em equilíbrio com o hospedeiro e, apenas quando esse equilíbrio é perturbado, a doença se instala. Esse desequilíbrio pode estar relacionado, por exemplo, com alterações nos níveis de mediadores inflamatórios, como as citocinas e quimiocinas, produzidas pelas células residentes e aquelas recrutadas em resposta à infecção persistente (CULLINAN *et al.*, 2003).

Algumas citocinas, como IL (interleucina)-1β, IL-6, IL-17, IFN (interferon)-γ e TNF (fator de necrose tumoral)-α, e quimiocinas, como CXCL8/IL-8 e CXCL10, são caracterizadas por suas atividades pró-inflamatórias (FENG & WEINBERG, 2006; GARLET, 2010), ao passo que outras citocinas, como IL-4 e IL-10, regulam negativamente tal resposta (MENG *et al*., 2007).

A citocina IL-17, por exemplo, é capaz de estimular células epiteliais, endoteliais e fibroblastos a produzirem IL-6, CXCL8/IL-8 e prostaglandina $E_2$ ($PGE_2$) (FOSSIEZ *et al*., 1996). A IL-17 também induz a expressão do ligante do receptor ativador do fator nuclear kappa-B (RANKL) por osteoblastos (KOTAKE *et al*., 1999), estimulando a diferenciação e ativação de osteoclastos, o que pode afetar a reabsorção óssea mediada por essas células (KOTAKE *et al*., 1999). Um número crescente de estudos tem demonstrado a presença de IL-17 nos tecidos periodontais, fluido crevicular gengival, saliva e plasma de pacientes com DP (JOHNSON, WOOD, SERIO, 2004; HONDA *et al.*, 2008; SCHENKEIN *et al*., 2010; OZÇAKA, NALBANTSOY, BUDUNELI, 2011). Dutzan *et al.*, (2009) observaram níveis mais altos de IL-17 em sítios ativos de destruição periodontal, enquanto Zhao *et al.*, (2011) verificaram que após o tratamento periodontal os níveis de IL-17 foram reduzidos.

O TNF-α, por sua vez, é uma citocina pró-infamatória que induz a secreção de colagenases, $PGE_2$ e IL-6, além da reabsorção óssea por ativação de osteoclastos (LOOS *et al*., 2005). O TNF-α está presente em níveis elevados no fluido crevicular gengival e nos tecidos periodontais de pacientes com periodontite (GRAVES & COCHRAN, 2003; GARLET *et al*., 2004; GRAVES, 2008). Estudos experimentais de DP demonstraram que TNF-α tem um papel importante na reação inflamatória, reabsorção do osso alveolar e na perda do ligamento

periodontal (GRAVES & COCHRAN, 2003; GARLET *et al*., 2007; QUEIROZ-JUNIOR *et al.*, 2011; 2012).

A IL-10 é uma citocina primariamente conhecida por suas funções anti-inflamatórias, produzida por células T regulatórias, células T auxiliares tipo 2, macrófagos e células B. A IL-10 atua inibindo a síntese de citocinas pró-infamatórias e estimulando a proliferação e a diferenciação das células B (ROUSSET *et al*., 1992). Essa citocina se encontra altamente expressa nos tecidos periodontais inflamados e tem sido associada com uma menor gravidade da doença (GARLET *et al*., 2004; 2006; COLIC *et al*., 2010). De fato, estudos com animais deficientes para IL-10 mostraram que esses são altamente susceptíveis à perda óssea alveolar induzida por *P. gingivalis* (SASAKI *et al*., 2004) e essa perda pode estar associada à redução da atividade de osteoblastos (CLAUDINO *et al*., 2010).

Em doenças inflamatórias como a DP, o que ocorre é um desequilíbrio entre mediadores pró e anti-inflamatórios em favor da inflamação, havendo posteriormente uma tendência de aumento dos mediadores anti-inflamatórios quando o processo se torna crônico (GARLET *et al*., 2010; KINANE, PRESHAW, LOOS, 2011). Esse equilíbrio é influenciado pelo tipo e carga de microrganismos nos tecidos periodontais, por doenças sistêmicas adquiridas que reduzem ou alteram a resistência orgânica e por fatores ambientais. Entretanto, existe um reconhecimento crescente sobre a influência do componente genético na susceptibilidade à periodontite (OFFENBACHER, 1996), sendo possível identificar genes que lhe conferem susceptibilidade. Acredita-se que os genes responsáveis pela resposta imune desempenham um papel crucial na patogênese da periodontite. Assim, o controle genético da expressão das citocinas pode afetar o aparecimento e a gravidade das DPs (TAYLOR *et al.*, 2004).

Como tem sido demonstrado que mecanismos genéticos podem ter impacto em desordens inflamatórias e devido a resultados conflitantes sobre esses eventos na periodontite somado a falta de dados sobre polimorfismos no gene da *IL-17* e DP, trabalhos nessa área poderão contribuir para o conhecimento sobre a patogênese dessa doença (GOMEZ; DUTRA E MOREIRA, 2009).

## 1.3 POLIMORFISMO GENÉTICO

O polimorfismo genético é a variação genética na sequência de alelos, na sequência de bases nucleotídicas ou na estrutura cromossômica, que ocorre com uma frequência maior que 1% na população (MILLER *et al*., 2001). O tipo de polimorfismo mais estudado é o de base única, conhecido como Polimorfismo de Nucleotídeo Único (*Single Nucleotide Polimorphism* - SNP), que consiste em uma variação de um nucleotídeo singular num sítio particular do genoma (TAYLOR *et al*., 2004).

A presença de um polimorfismo pode implicar em mudança no código genético, levando a alterações no genótipo (sequência de bases), afetando ou não o fenótipo. Ou seja, um polimorfismo pode influenciar, por exemplo, o nível de secreção de substâncias e levar a variações nas respostas imunológicas e inflamatórias individuais frente a uma agressão bacteriana (TAYLOR *et al.*, 2004).

Polimorfismos funcionais, relacionados à diferença interindividual na síntese e secreção de proteínas, têm sido associados a doenças que têm uma patogênese inflamatória (PARKHILL *et al*., 2000). Sendo assim, a maioria dos estudos nesta área tem focado em genes que codificam citocinas e receptores de moléculas inflamatórias, inclusive na doença periodontal (TAYLOR *et al.*, 2004; BRETT *et al*., 2005).

A busca de polimorfismos genéticos que possam interferir na susceptibilidade individual de algumas doenças tem encontrado resultados contraditórios entre as populações (LAINE *et al*.,2010) . A explicação para tais achados deve se ao fato de que populações geográfica e etnicamente distintas exibem um componente genético diferenciado entre elas, assim como a possível presença de fatores de risco diferentes nas áreas estudadas, o que provavelmente pode acarretar em resultados distintos.

## 1.4 POLIMORFISMOS GÉNETICOS E DP

Os primeiros relatos associando periodontite e fatores genéticos vieram da observação de que se encontravam, com muita freqüência, irmãos de portadores de periodontite também desenvolvendo doença periodontal (BOUGHMAN, 1992). Nesta época surgiram também os primeiros estudos em gêmeos, correlacionando os aspectos genéticos à ocorrência de periodontite (MICHALOWICZ, 1994; 1999).

Segundo Figueiredo (2001), não existem síndromes genéticas que possam ser usadas como marcadores de risco para a periodontite crônica. Porém, substâncias pró-inflamatórias podem ser consideradas potenciais responsáveis por uma resposta inflamatória exacerbada, a qual pode explicar a transição de uma gengivite para uma periodontite. Entretanto, ainda não está bem estabelecido na literatura quais os genes que regulam mediadores inflamatórios que poderiam funcionar como modificadores das DPs.

Nesse contexto, sabe-se, por exemplo, que os níveis de IL-17A e IL-17F encontram-se aumentados nos sítios com maior profundidade de sondagem de pacientes com DP (VERNAL *et al*., 2005; OHYAMA *et al*., 2009) e já foi demonstrado que o alelo ancestral (A) do polimorfismo de *IL-17A (-197 A/G)* está associado à maior produção desta molécula (ESPINOZA *et al*., 2011). Por outro lado, a variante alélica (C) do polimorfismo de *IL-17F (+7488 T/C)* tem sido associada à menor produção da mesma (KAWAGUCHI *et al*., 2006). Os polimorfismos dos genes que regulam a produção de IL-17A e IL-17F têm sido estudados e relacionados com artrite reumatóide (NORDANG *et al*. 2009), porém, seu envolvimento na DP ainda não está esclarecido. O polimorfismo do gene *IL-10* pode modular a sua expressão, diminuindo sua produção e consequentemente alterando sua atividade anti-inflamatória. Há alguns estudos mostrando a relação do polimorfismo -1087 da IL-10 com a maior susceptibilidade à doença periodontal (BERGLUNDH *et al.*, 2002; SCAREL-CAMINAGA *et al.*, 2004; SUMER *et al.*, 2007; CULLINAN *et al.*, 2008; ATANASOVSKA-STOJANOVSKA *et al.*, 2012), enquanto outros estudos refutam essa ligação (KINANE *et al.*, 1999; YAMAZAKI *et al.*, 2001; GONZALES *et al.*, 2002; BRETT *et al.*, 2005; BABEL *et al.*, 2006, TERVONEN *et al.*, 2007; MOREIRA *et al*., 2009; GUROL *et al.*, 2011).

Outro gene de interesse que tem sido largamente estudado é o gene do TNF-α. A presença do alelo *-308A* está associada com a maior produção de TNF-α (ABRAHAM & KROEGER, 1999). Além disso, tem sido demonstrado que o alelo A pode aumentar o risco de várias desordens infecciosas e inflamatórias (WILSON *et al*., 1995), incluindo DP (GALBRAITH *et al*., 1999; LIN *et al*., 2003; RICCI *et al*., 2011). Entretanto, outros estudos

que compararam a presença de polimorfismo de *TNFA (-308 G/A)* em indivíduos clinicamente saudáveis e com periodontite agressiva ou crônica não mostraram diferenças significativas (CRAANDIJK *et al.*, 2002; FASSMANN *et al*., 2003; FOLWACZNY *et al.*, 2004; SAKELLARI *et al.*, 2006; MENEZES & COLOMBO, 2007; MOREIRA *et al*., 2009; COSTA *et al.*, 2010).

Tendo em vista o importante papel das citocinas IL-17, IL-10 e TNF-α na patogênese da doença periodontal e os dados conflitantes na literatura sobre a possível associação destes polimorfismos genéticos com uma maior gravidade da DP, torna-se importante investigar a presença desses polimorfismos em pacientes com DP. Além disso, a maioria dos estudos não avalia as possíveis alterações fenotípicas associadas a esses polimorfismos. Deste modo, é importante relacionar a presença dos polimorfismos com possíveis modificações do padrão inflamatório nesses pacientes, além da relação com as condições clínicas periodontais desses indivíduos.

# 2 OBJETIVOS

## 2.1 Objetivo Geral

Avaliar a possível relação de polimorfismos nos genes *IL-17A*, *IL-17F*, *IL-10* e *TNFA* com o fenótipo da doença periodontal.

## 2.2 Objetivos Específicos

1. Comparar a ocorrência dos polimorfismos genéticos entre pacientes com periodontite crônica e indivíduos sem a doença (controles).
2. Realizar análise do infiltrado inflamatório nos tecidos gengivais obtidos de pacientes com doença periodontal e controles;
3. Avaliar a expressão de citocinas (IL-10, IL-17, TNF-α e IFN-γ) e quimiocinas (CXCL10, CXCL8/IL-8) no soro e nos tecidos gengivais obtidos de pacientes com doença periodontal e controles;
4. Correlacionar os níveis de expressão de mediadores inflamatórios no sangue periférico e tecidos gengivais com parâmetros clínicos da doença;
5. Correlacionar os dados clínicos e parâmetros inflamatórios com os diferentes genótipos dos genes *IL-17A, IL-17F, IL-10 e TNFA*.

## 3 METODOLOGIA E RESULTADOS

A metodologia do presente trabalho, bem como o capítulo de resultados serão apresentados em formato de artigos científicos:

O primeiro artigo *"Association between Polymorphisms in Interleukin-17A and -17F Genes and Chronic Periodontal Disease"* foi publicado na revista "*Mediators of Inflammation*", volume 2012, páginas 1-9, 2012.

O segundo artigo "*Polymorphisms of IL-10 and TNFA genes and inflammation status in chronic periodontitis"* está em fase de preparação.

# 3.1 Artigo I

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 846052, 9 pages
doi:10.1155/2012/846052

*Clinical Study*

# Association between Polymorphisms in Interleukin-17A and -17F Genes and Chronic Periodontal Disease

**Jôice Dias Corrêa,[1] Mila Fernandes Moreira Madeira,[2] Renata Gonçalves Resende,[1] Jeane de Fátima Correia-Silva,[1] Ricardo Santiago Gomez,[1] Danielle da Glória de Souza,[2] Mauro Martins Teixeira,[3] Celso Martins Queiroz-Junior,[1,4] and Tarcília Aparecida da Silva[1]**

[1] *Departmento de Clínica, Patologia e Cirurgia Odontológicas, Faculdade de Odontologia, Universidade Federal de Minas Gerais, 31.270-901 Belo Horizonte, MG, Brazil*
[2] *Departmento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31.270-901 Belo Horizonte, MG, Brazil*
[3] *Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31.270-901 Belo Horizonte, MG, Brazil*
[4] *Departamento de Clínica, Patologia e Cirurgia Odontológicas, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Avendia Antônio Carlos 6627, 31.270-901 Belo Horizonte, MG, Brazil*

Correspondence should be addressed to Celso Martins Queiroz-Junior, cmqj@yahoo.com.br

Received 26 September 2012; Revised 7 November 2012; Accepted 7 November 2012

Academic Editor: Fábio Santos Lira

Copyright © 2012 Jôice Dias Corrêa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Objective*. Interleukin-17 (IL-17) is a cytokine that induces neutrophil recruitment and the release of inflammatory mediators in several inflammatory conditions; nevertheless, the involvement of IL-17 gene polymorphisms in chronic periodontitis (CP) has not been addressed yet. Our aim was to evaluate the association between periodontal status and the polymorphisms IL-17A G197A and IL-17F C7488T in subjects with CP along with their impact on levels of inflammatory mediators. *Material and Methods*. Genomic DNA was obtained from 30 CP patients and 30 healthy controls (HCs). IL-17A G197A and IL-17F C7488T polymorphisms were determined using PCR-RFLP. Serum and periodontal tissues were collected and processed for ELISA, myeloperoxidase (MPO), and/or microscopic analysis. *Results*. The frequencies of genotypes in the CP group were significantly different from those of HC. Odds ratio indicated that increased risks for CP were associated with the -197A allele, not with the -7488T allele. In addition, the -197A allele was correlated with worse clinical parameters, higher MPO activity, and increased expression of inflammatory mediators (IL-17A and IL-8) than the other genotypes. *Conclusions*. These results indicate that the IL-17A -197A allele is associated with increased risk for CP, likely because this genotype relates to the enhanced inflammation in periodontal tissues.

## 1. Introduction

Periodontitis is an inflammatory disease that affects the tooth-supporting tissues. It is considered one of the most significant causes of tooth loss in humans and may associate with systemic diseases, such as diabetes and arthritis [1–4]. Pathogens of the subgingival bacterial biofilm are essential for the initiation and progression of periodontitis. Nevertheless, disease results from the host inflammatory reaction that primarily mediates tissue damage [2, 5, 6]. For many years, the pathogenesis of periodontitis was classically viewed as involving an immunological Th1/Th2 paradigm. According to this view, the tissue destructive Th1 cells and cytokines would arise in the early period of the disease, while the tissue protective Th2 cells and cytokines would arise in the late phase [2, 6, 7]. However, in several clinical contexts, the Th1/Th2 balance/imbalance is not sufficient to explain the progression and/or remission of periodontitis observed in patients [7].

In 2005, the Th17 subset of $CD4^+$ T cells was identified [8] and added greater complexity to Th function. Th17 cells are generally considered to be proinflammatory, in particular

through the production of the cytokines interleukin-17A (IL-17A) and IL-17F. These cells and cytokines have been associated with the pathogenesis of an extensive list of autoimmune and inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, psoriasis [9–11], and periodontitis [12–15]. The majority of studies have reported increased IL-17 levels associated with the development of chronic periodontitis (CP) [14–18]. In experimental models, Th17 is suggested to play a role in the development of disease [17]. In humans, elevated levels of IL-17 have been reported in patients with CP, but it is not clear why such elevated levels are found in these patients [14, 15, 17]. The study of genetic polymorphisms in CP has received increasing attention lately as they describe the contribution of genes to disease progression [18].

Allelic variants of cytokine genes are typically related to either higher or lower production of these molecules [18, 19]. In this regard, it is reasonable to hypothesize that genetic variation affecting the expression or activity of IL-17 may influence the susceptibility and severity of periodontitis. IL-17A and IL-17F genes are mapped on the same chromosome at position 6p12 [20], and the polymorphisms of IL-17A G197A (rs2275913) and IL-17F C7488T (rs763780) have recently been associated with higher susceptibility to rheumatoid arthritis [21] and ulcerative colitis [22]. Nevertheless, the possible involvement of IL17 gene polymorphisms in CP has not been evaluated yet.

The purpose of the current study was to determine whether the IL-17A G197A and IL-17F C7488T polymorphisms were associated with increased susceptibility to periodontitis. We studied the association between each single nucleotide polymorphism (SNP) and the clinicopathological features of CP and local and systemic production of inflammatory mediators.

## 2. Material and Methods

*2.1. Subjects and Sample Collection.* Gingival tissue samples were obtained from periodontal tissues resected during periodontal surgery from 30 patients with CP who attended the Periodontal Clinic, School of Dentistry, Universidade Federal de Minas Gerais (UFMG). All patients had a previous history of CP and were diagnosed according to previously described criteria, including >35 years of age, no marked familial aggregation, and variable distribution of periodontal destruction [23, 24]. The inclusion criteria were subjects with attachment loss >5 mm at more than one tooth, more than three sites of probing depth >6 mm, and lesions distributed at more than two teeth in each quadrant; these subjects were diagnosed with CP. Exclusion criteria were aggressive periodontitis, use of antibiotic, anti-inflammatory and/or immunosuppressive medications in the 6 months preceding the research, and/or any systemic diseases (i.e., immunologic and autoimmune disorders, diabetes mellitus). Thirty periodontally healthy patients subjected to fully impacted third molar extraction, age- and gender-matched to the CP group, comprised the healthy control group (HC). In the current study, the individuals have not been stratified in ethnic groups based on skin color, race, or geographic origin due to the significant miscegenation among Brazilian population [25, 26].

Both groups of patients, CP and HC, were also subjected to periodontal examination including determination of probing depth (PD), clinical attachment loss (CAL), bleeding on probing (BOP), and tooth mobility. The BOP was considered positive if bleeding occurred within 30 seconds after probing [27]. Measurements were performed full mouth at 6 sites per tooth (mesiobuccal, mid buccal, distobuccal, mesiolingual, mid lingual, and distolingual). At the time of the examination, a peripheral blood sample was taken from each patient and processed for polymorphism determination and serum obtainment.

Written informed consent was obtained from all patients. This study protocol was approved by the local Institutional Ethics Committee (324/08).

*2.2. Inflammatory Infiltrate Evaluation.* Gingival tissue samples were also fixed in 10% buffered formalin, embedded in paraffin wax, and cut longitudinally (3 $\mu$m). The sections were deparaffinized, rehydrated, and stained with H&E for the evaluation of the inflammatory infiltration. Inflammatory cells were counted in four fields in two independent sections (total evaluated area: ~1 $mm^2$), using light microscope (Axioskop 40 ZEISS; Carl Zeiss, Gottingen, Germany) at 400x magnification. Data were expressed as total of inflammatory cells/field.

*2.3. ELISA.* The concentrations of the cytokines IL-17A, IL-17F, interferon (IFN)-$\gamma$ and tumor necrosis factor (TNF)-$\alpha$, and the chemokines CXCL10 and IL-8 were measured in gingival tissues and serum by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (R&D Systems, Minneapolis, MN, USA).

The assay was performed according to the manufacturer's instructions. In brief, tissue samples have been weighed, mechanically homogenized in buffer solution (0.4 mM NaCl, 10 mM $NaPO_4$, pH 7.4) containing inhibitors of proteases (0.1 mM phenylmethylsulfonyl—PMSF fluoride—0.1 mM benzethonium chloride, 10 mM EDTA and 0.01 mg/mL aprotinin A) and Tween 20 (0.05%), pH 7.4 (normalization: 1000 $\mu$L of solution for 100 mg of wet tissue), and centrifuged (10,000 rpm, 10 min. 4°C).

Each cytokine was detected by an anticytokine horseradish peroxidase-labelled monoclonal antibody. The OPD (*o*-phenylenediamine dihydrochloride, Sigma-Aldrich, Saint Louis, MO, USA) peroxidase substrate kit was used to determine the amount of horseradish peroxidase bound to each well. The reaction was stopped by the addition of 1 M sulfuric acid ($H_2SO_4$). The plates were read at 492 nm. The data were determined using a standard curve prepared for each assay and expressed as picograms of cytokine/chemokine per 100 mg of tissue or mL of serum.

*2.4. Myeloperoxidase.* Gingival tissue samples were also used for determination of myeloperoxidase (MPO) activity, a neutrophil enzyme marker, as previously described [28].

After processing for ELISA, the remaining tissue pellets were subjected to hypotonic lysis: 0.2% NaCl solution for 30 s followed by addition of an equal volume of a solution containing 1.6% NaCl and 5% glucose. After further centrifugation, the pellets were resuspended in 0.05 M sodium phosphate buffer (pH 5.4) containing 0.5% hexa-1,6-bisdecyltrimethylammonium bromide (HTAB, Sigma-Aldrich). The suspensions were freeze thawed three times and finally centrifuged at 10,000 rpm for 10 min at 4°C. MPO activity in 25 $\mu$L of the resulting supernatant was assayed by adding 25 $\mu$L of 3,3′-5,5′-tetramethylbenzidine (TMB, Sigma-Aldrich) prepared in dimethylsulfoxide (DMSO, Merck, NJ, USA, 1.6 mM), and 100 $\mu$L of $H_2O_2$ (0.002%, v/v) was diluted in phosphate buffer (pH 5.4) containing 0.5% HTAB. The assay was performed in a 96-well microplate incubated for 5 min at 37°C. The reaction was stopped by adding 100 $\mu$L of 4 M $H_2SO_4$ and quantified colorimetrically at 450 nm in a spectrophotometer.

*2.5. DNA Isolation and Genotyping Analysis.* Total genomic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer instructions. Quality, integrity and quantity of DNA were analyzed by Nanodrop spectrophotometer (Thermo Scientific-GE). Single nucleotide polymorphisms (SNPs) of the IL-17A (rs2275913) and IL-17F (rs763780) genotyping were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Table 1).

The PCR amplification was performed in a total volume of 25 $\mu$L mixture containing 100 ng genomic DNA, 1.0 $\mu$M of each primer 20 $\mu$L of Premix buffer (Phoneutria Biotecnologia, Belo Horizonte, Brazil). According to the manufacturer, the Premix buffer contained 50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.1% Triton X-100, 1.5 mM MgCl2, deoxynucleoside triphosphates, and 1.25 units of Taq DNA polymerase.

PCR products were digested overnight at 37°C with XagI (Fermentas) for IL-17A G197A. The products of IL-17A G197A and IL-17F C7488T were viewed in a 6.5% polyacrylamide gel electrophoresis stained with silver.

*2.6. Data Analysis.* Data were expressed as mean ± standard deviation (SD). All data were analyzed using SPSS 17 (SPSS Inc., Chicago, IL, USA). Chi-square test analysis was used to test for deviation of genotype frequencies from Hardy-Weinberg equilibrium.

The levels of cytokines in periodontal tissues and the frequency of gene polymorphisms were compared by the Student's $t$-test and chi-square test. Odds ratios were calculated for the minor allele at each SNP. $P$ values < 0.05 were considered statistically significant.

## 3. Results

*3.1. Differences between Healthy Controls (HCs) and Chronic Periodontitis (CP) Subjects.* The demographic characteristics of the studied population are presented in Table 2. The age and gender were not significantly different between groups.

Table 1: Primer sequences for each gene.

| | Primers |
|---|---|
| IL-17A | Sense 5′-AACAAGTAAGAATGAAAAGAGGACATGGT-3′ |
| | anti-sense 5′-CCCCCAATGAGGTCATAGAAGAATC-3′ |
| IL-17F | Sense 5′-GTTCCCATCCAGCAAGAGAC-3′ |
| | anti-sense 5′-AGCTGGGAATGCAAACAAAC-3′ |

The frequency of smoker subjects in the studied sample was 3.3% in HC ($n = 1$) and 20% in CP ($n = 6$) groups. These patients did not present significant differences in the clinical parameters and production of inflammatory mediators when compared with nonsmokers ($P > 0.05$; data not shown). In this regard, data from smoker and nonsmoker subjects were grouped and presented together.

The clinical features PD, CAL, BOP, and tooth mobility (not shown) were significantly higher in the group CP than those in the group HC ($P < 0.0001$) (Table 2).

Besides clinical features, we also evaluated the levels of the cytokines IL-17A, IL-17F, IFN-$\gamma$ and TNF-$\alpha$, and the chemokines IL-8 and CXCL10 in periodontal tissues and/or serum of HC and CP subjects. Overall, levels of inflammatory mediators were increased in tissue and serum of CP patients when compared to the HC group, with the exception of IL-17F in serum ($P > 0.05$) (Figures 1(a)–1(c)). Levels of IFN-$\gamma$ (HC: 45 ± 23 pg/100 mg tissue; CP: 131 ± 98 pg/100 mg tissue; $P < 0.0001$), TNF-$\alpha$ (HC: 21 ± 9 pg/100 mg tissue; CP: 117 ± 49 pg/100 mg tissue; $P < 0.0001$), and CXCL10 (HC: 15 ± 4 pg/100 mg tissue; CP: 34 ± 27 pg/100 mg tissue; $P = 0.003$) in periodontal tissues were also greater in CP patients than those in controls. Moreover, the inflammatory infiltrate in the gingival tissue, characterized by mixed polymorpho- and mononuclear cells, with a predominance of mononuclear leukocytes, was significantly higher in the CP than in the HC group (HC: 18 ± 8 inflammatory cells/field; CP: 88 ± 20 inflammatory cells/field; $P < 0.0001$). MPO activity was also significantly greater in CP than in HC subjects ($P < 0.0001$) (Figure 1(d)).

Frequencies of polymorphisms (IL-17A G197A and IL-17F C7488T genotypes) were investigated in blood samples of HC and CP subjects (Table 3). The frequency of these genotypes agreed with the Hardy-Weinberg equilibrium ($P > 0.05$). The mean ages of the control group (AA: 44.2; AG: 47.8; GG: 45.2 years old) and patients with CP (AA: 40.0; AG: 42.0; GG: 40.8 years old) versus genotype did not present statistical differences ($P > 0.05$). The IL-17A genotypes of the CP group (GG 20%; GA 30% and AA 50%) were significantly different from the frequencies observed in the HC group (GG 59.26%; GA 14.81% and AA 25.92%) ($\chi^2 = 9.307$; $P = 0.01$). The overall A carrier subjects (GA or AA) were associated with increased risk for periodontal disease when compared with GG carriers (OR 3.00, 95% CI: 1.34–6.67, $P = 0.001$). In contrast, the distribution of the IL-17F C7488T polymorphism was similar among the groups ($\chi^2 = 0.954$; $P = 0.62$) (Table 3).

*3.2. Association between the IL-17A G197A and IL-17F C7488T Polymorphisms and Clinical Periodontal Parameters.*

Table 2: Demographic and clinical features of the studied subjects.

| | HC ($n = 30$) | CP ($n = 30$) | $P$ value |
|---|---|---|---|
| Age (SD; range) | 40.5 (8.1; 26–52) | 45.5 (8.7; 37–61) | 0.97 |
| Gender (% F) | 60.86 | 50.00 | 0.44 |
| Ethnic origin | Brazilian mixed population | | |
| PD (SD) | 2.50 (0.8) | 4.52 (0.19)* | <0.0001 |
| CAL (SD) | 2.65 (0.15) | 5.74 (0.17)* | <0.0001 |
| BOP (SD) | 2.0 (0.4) | 31.17 (4.02)* | <0.0001 |

HC: healthy controls, CP: chronic periodontitis, SD: standard deviation, PD: probing depth, CAL: clinical attachment loss, BOP: and bleeding on probing.
*Significantly higher than control ($\chi^2$ test or Student's $t$-test).

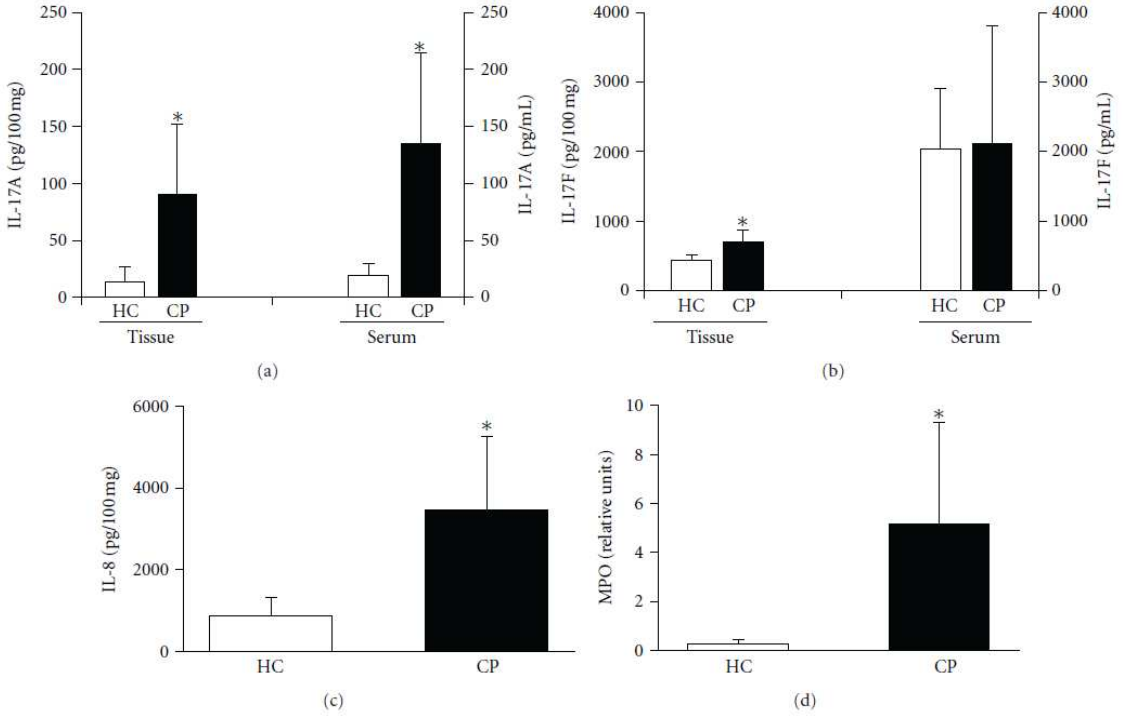


Figure 1: Levels of (a) IL-17A and (b) IL-17F in the gingival tissue and serum samples from CP and HC subjects. (c) Levels of IL-8 and (d) MPO activity in gingival tissue samples from CP and HC subjects. *Statistically significant difference at $P < 0.05$. HC: healthy control, CP: chronic periodontitis, and MPO: myeloperoxidase.

In view of the results indicating higher frequency of AG/AA alleles among the IL-17A G197A genotypes of patients with periodontitis, we further investigated whether some of these polymorphisms were associated with worse clinical periodontal parameters. As shown in Table 4, the intragroup comparison of the three IL-17A G197A genotypes indicated that PD and CAL, but not BOP, were significantly higher in AG and AA subjects than in patients with the GG genotype. Indeed, there was a significant correlation between the levels of IL-17A and PD in subjects with the genotype AA (data not show). In contrast, the IL-17F C7488T genotypes did not affect the clinical features of periodontally affected patients (Table 4).

*3.3. Association between the IL-17A G197A and IL-17F C7488T Polymorphisms and Inflammatory Features.* The association between IL-17 gene polymorphisms and the presence of inflammatory mediators in periodontal tissues and serum was also investigated. As shown in Figure 2(a), the levels of IL-17A in periodontal tissues from cases were not different when comparing the IL-17A G197A genotypes to each other. However, the serum levels of IL-17A were higher in subjects with the allele A than in subjects with the alleles GG (Figure 2(b)). In patients without CP, there were no differences in the levels of IL-17A in the gingival tissue or serum among the genotypes (gingival tissue: AA: 14 ± 9 pg/100 mg tissue; AG: 17 ± 17 pg/100 mg tissue; GG: 8

Table 3: Genotypes of IL-17 polymorphisms in patients with chronic periodontitis (CP) and healthy controls (HCs).

| Genotype | HC (%) | CP (%) | *P* value | OR (95% CI) |
|---|---|---|---|---|
| IL-17A G197A | | | | |
| AG/AA | 40.73 | 80.00* | 0.001 | 3.00 (1.34–6.67) |
| AA | 25.92 | 50.00* | 0.002 | 3.03 (1.34–6.86) |
| AG | 14.81 | 30.00* | 0.014 | 2.94 (1.24–7.00) |
| GG | 59.26 | 20.00* | 0.001 | 1 |
| IL-17F C7488T | | | | |
| CT/TT | 73.26 | 63.4 | 0.310 | 1.30 (0.80–2.15) |
| CT | 16.66 | 20.0 | 0.350 | 0.47 (0.09–2.30) |
| TT | 56.66 | 43.4 | 0.400 | 0.58 (0.18–2.04) |
| CC | 23.33 | 36.6 | 0.450 | 1 |

OR: odds ratio and CI: confidence interval.
*Significantly different from control $P < 0.05$ ($\chi^2$ test).

Table 4: Association between IL-17A G197A and IL-17F C7488T polymorphisms and clinicopathological features of chronic periodontitis.

| Genotype | *n* | PD (mm) | *P* value | CAL (mm) | *P* value | BOP (%) | *P* value |
|---|---|---|---|---|---|---|---|
| IL-17A G197A | | | | | | | |
| AA/AG | 24 | 4.82* | 0.012 | 6.00* | 0.004 | 33.40 | 0.31 |
| AA | 15 | 4.58* | 0.005 | 5.77* | 0.01 | 39.45 | 0.14 |
| AG | 9 | 5.29* | 0.007 | 6.429* | 0.004 | 22.14 | 1.00 |
| GG | 6 | 3.30 | | 4.70 | | 23.75 | |
| IL-17F C7488T | | | | | | | |
| CT/TT | 19 | 4.86 | 0.11 | 5.571 | 0.14 | 25.89 | 0.27 |
| TT | 6 | 4.91 | 0.20 | 5.63 | 0.16 | 25.24 | 0.25 |
| CT | 13 | 4.67 | 0.18 | 6.67 | 0.59 | 28.30 | 0.71 |
| CC | 11 | 5.25 | | 6.25 | | 37.33 | |

PD: probing depth, CAL: clinical attachment loss, and BOP: bleeding on probing.
*Significantly different from GG or CC genotype ($P < 0.05$, Student's *t*-test).

± 3 pg/100 mg tissue; $P > 0.05$; serum: AA: 26 ± 10 pg/100 mg tissue; AG: 14 ± 1 pg/100 mg tissue; GG: 17 ± 10 pg/100 mg tissue; $P > 0.05$). Interestingly, the levels of the chemokine IL-8 were increased in tissues from CP patients with the allele A (Figure 2(c)), while the levels of CXCL10 were higher only in AG carriers (AA: 35 ± 29 pg/100 mg tissue; AG: 60 ± 7 pg/100 mg tissue; GG: 15 ± 2 pg/100 mg tissue; $P = 0.004$), as also occurred for MPO activity (Figure 2(d)). The histological findings indicated a mixed polymorpho- and mononuclear inflammatory infiltrate equally distributed among the polymorphisms groups (AA: 95 ± 11 inflammatory cells/field; AG: 87 ± 25 inflammatory cells/field; GG: 97 ± 5 inflammatory cells/field; $P > 0.05$).

The levels of IL-17F were not different, neither in periodontal tissues nor in serum, among the IL-17F C7488T genotypes (Figures 3(a) and 3(b)). The same occurred with levels of IL-8 and MPO activity, which were not different among the groups (Figures 3(c) and 3(d)). In regard to inflammatory infiltration, there were no differences among the three genotypes (CC: 102 ± 11 inflammatory cells/field; CT: 115 ± 23 inflammatory cells/field; TT: 82 ± 24 inflammatory cells/field; $P > 0.05$).

## 4. Discussion

Several experimental and clinical studies have shown that IL-17 levels are elevated in diseased human periodontal tissues and may play a destructive role in experimental models of periodontal disease [3, 14–17, 29, 30]. In the current study, we investigated the involvement of IL-17 genes polymorphisms in CP. Our results confirm that IL-17 levels are elevated in periodontal tissues of CP patients. More importantly, we show for the first time that polymorphisms of IL-17A, specially the SNP involving the allele A, are associated with the clinical and inflammatory parameters of disease. There are increased levels of IL-17A in the serum of allele A carriers, and this is accompanied by an increase of IL-8 and MPO activity in periodontal tissues.

The high levels of IL-17 in gingival crevicular fluid and periodontal tissues of patients with CP have been shown to associate with periodontal tissue damage [14, 17], but also seem to be relevant to control excessive microbial replication and, hence, disease [31]. Our study showed that the clinical parameters of CP were associated with increased levels of IL-17A and IL-17F in gingival tissues, in agreement with

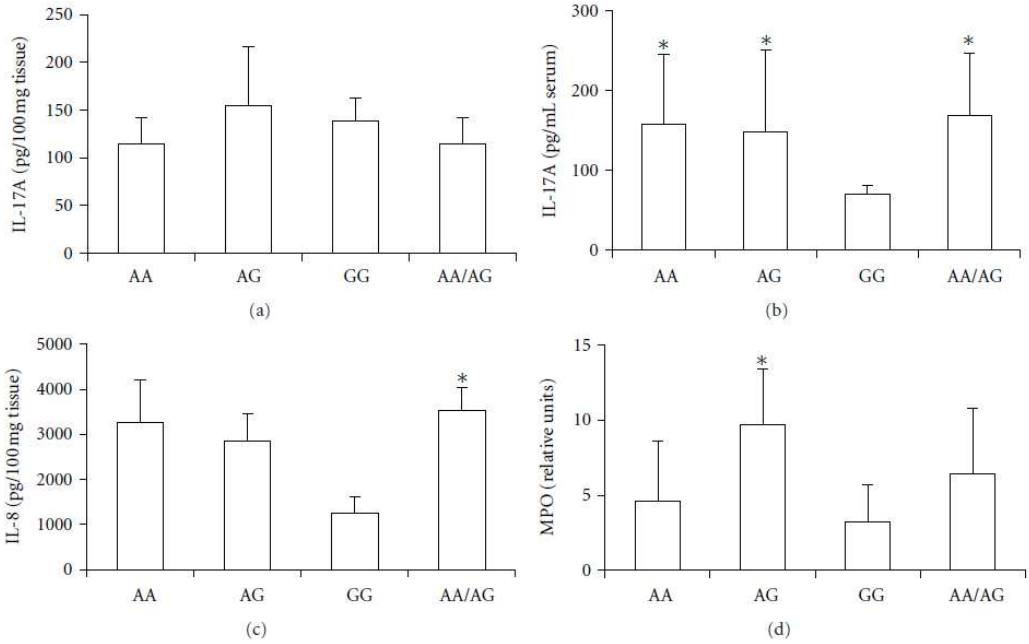


FIGURE 2: Levels of inflammatory mediators in chronic periodontitis patients according to each IL-17A G197A genotype. (a) Levels of IL-17A in gingival tissues and (b) serum. (c) Levels of IL-8 and (d) MPO (myeloperoxidase) activity in gingival tissue samples. *Statistically significant difference ($P < 0.05$) comparing with the genotype GG.

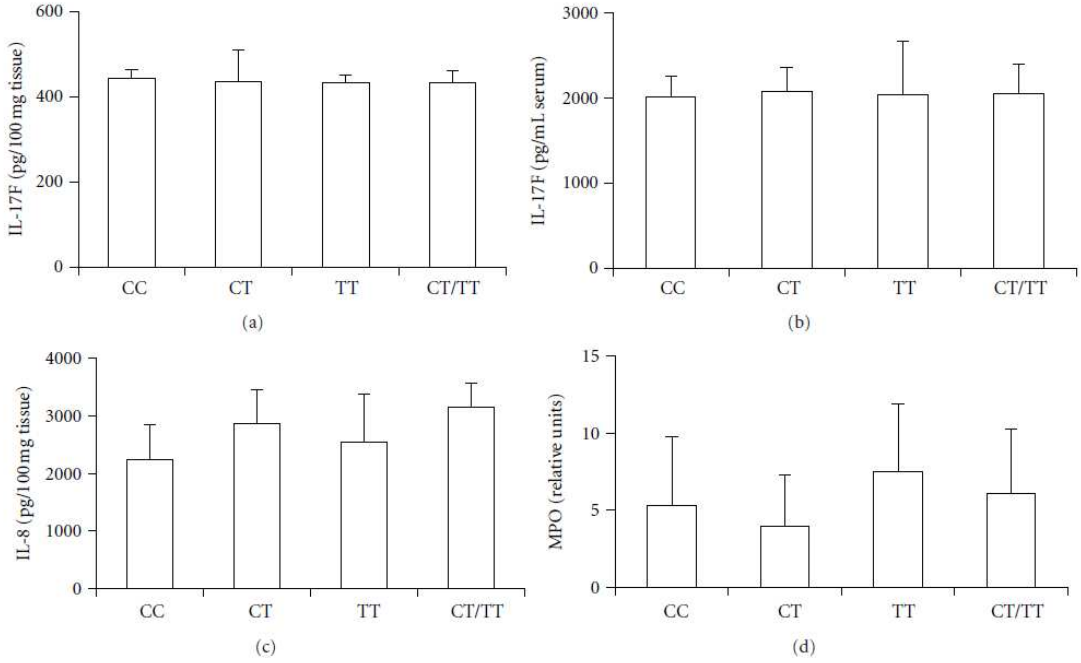


FIGURE 3: Levels of inflammatory mediators in chronic periodontitis patients according to each IL-17F C7488T genotype. (a) Levels of IL-17F in gingival tissues and (b) serum. (c) Levels of IL-8 and (d) MPO (myeloperoxidase) activity in gingival tissue samples.

previous reports [14, 17, 30]. In contrast, only the levels of IL-17A, not IL-17F, were enhanced in the serum of CP patients. This is in line with studies showing increased serum concentrations of IL-17A, mainly for patients with aggressive periodontitis [1, 32]. As CP is marked by recurrent phases of remission and activation, IL-17 may be related to the destructive period of the disease given that this cytokine has already been shown to be overexpressed in active periodontal sites [33]. Along with the high levels of IL-17 in tissue and serum, the MPO activity and IL-8 levels were higher in periodontal tissues of CP patients than in healthy subjects, as well as the presence of neutrophils in the diseased gingiva. Despite the nonmechanistic nature of the current data, IL-17 has already been shown to present a prominent role in the activation and recruitment of neutrophils to inflammatory sites [16], and MPO and IL-8 have been reported to be correlated with worse clinical status of periodontitis [34, 35]. These findings suggest an active inflammatory scenario, with increased expression of IL-17 in CP patients and the probable involvement of IL-17 in the increased influx of neutrophils to periodontal affected sites.

After detecting high levels of IL-17 in CP, we analyzed the frequency of IL-17 polymorphisms in CP and HC subjects. The investigation of gene polymorphisms in CP has long been conducted, likely because they present a role in immune responses, tissue destructive mechanisms, and metabolic processes [36]. Several research groups have studied the association between CP and polymorphisms of candidate genes, including pro- and anti-inflammatory cytokines, such as IL-1$\beta$, IL-4, IL-6, IL-10, and TNF-$\alpha$ [36–40]. Most studies recognized the proinflammatory gene cluster polymorphisms, especially TNF-$\alpha$ and IL-1, as some of the best candidates associated with the induction and severity of CP [36, 41]. In the current investigation, the IL-17F C7488T polymorphism was not different in healthy subjects and CP patients. Despite some evidence suggesting that IL-17F may play a role in periodontal bone destruction [14] and also in the stimulation of some cytokines and chemokines, including IL-6, IFN-$\gamma$, and CXCL10 in inflammatory conditions [42], our data have shown that neither the clinical parameters nor the levels of inflammatory mediators in periodontal tissues were influenced by the different IL-17F C7488T genotypes. In contrast, when evaluating IL-17A, we detected a significant difference in the distribution of genotypes for the polymorphism IL-17A G197A comparing subjects with and without periodontitis. CP patients presented increased frequencies of AA and AG genotypes, and the presence of the allele A significantly increased the risk for CP. These findings are in line with previous studies demonstrating the relationship between IL-17 polymorphisms, especially the allelic polymorphic A, and chronic inflammatory diseases, including rheumatoid arthritis [21], Behçet's disease [43], ulcerative colitis [22], and gastric and breast cancer [44, 45]. They are also in line with the previously described role of polymorphisms of proinflammatory cytokine genes during CP [36, 41]. Nevertheless, it seems reasonable to remember that gene mutations alone are neither sufficient nor necessary to explain disease phenotype, although they may contribute significantly to environment and life-style parameters in the outcome of CP.

In the current study, we show that IL-17A G197A allele A carriers presented higher serum levels of IL-17A, worse clinical periodontal parameters, and increased neutrophil activity (MPO activity and IL-8 levels) when compared with the GG genotype. Although not mechanistically conclusive, these findings seem to be in line with the hypothesis of a neutrophil-mediated tissue injury associated with increased levels of IL-17A during CP, which has recently been suggested as a target mechanism for tissue destruction in experimental conditions of periodontitis associated with old age [16, 46]. Th17 lymphocytes have already been shown to be present and play a significant role in CP [47]. IL-17A can directly or indirectly (via production of chemokines) chemoattract neutrophils [48] and enhance the activity of proteolytic enzymes such as neutrophil protease and myeloperoxidase [31]. Moreover, IL-17A can stimulate the expression of bone resorption mediators, such as RANKL [49] and induce the direct differentiation of bone resorptive cells [50] and the production and release of a large range of inflammatory mediators [51], such as TNF-$\alpha$, IFN-$\gamma$, and the chemokines CXCL10 and IL-8, all detected here. Indeed, a recent study showed that IL-17 can enhance CXCL10 production *in vitro* by TNF-$\alpha$- and IFN-$\gamma$-stimulated human gingival fibroblasts [52] and may induce IL-8 production by gingival fibroblasts [53]. In line with these biological functions, the increased production of IL-17 seems to be predictive of tissue destruction in inflammatory conditions, such as in rheumatoid arthritis [54]. Altogether, these data point that IL-17A G197A polymorphism, especially carriers of the allele A, might be associated with increased expression of IL-17A, recruitment of neutrophils, and worse clinical conditions in CP patients.

In conclusion, this is the first study to show that the IL-17A G197A polymorphism is related to CP in a convenient sample of Brazilian patients. Although this is a relatively small sample, the presence of the allele A in IL-17A-197 polymorphism was associated with worse clinical and inflammatory periodontal parameters. It is not simple to determine in humans the mechanisms underlying the greater risk of disease in carriers of the IL-17A-197 allele A. However, our study suggests that the latter polymorphism may contribute to disease by regulating IL-17A production and, probably, the consequent release of inflammatory and bone destructive mediators. It is, therefore, suggested that IL-17A might be an interesting target for development of new therapies for periodontal disease, an assertion that needs testing in further cohorts and clinical trials.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq

(Conselho Nacional de Desenvolvimento Científico e Tecnológico), and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), Brazil.

## References


[1] P. M. Duarte, M. Da Rocha, E. Sampaio et al., "Serum levels of cytokines in subjects with generalized chronic and aggressive periodontitis before and after non-surgical periodontal therapy: a pilot study," *Journal of Periodontology*, vol. 81, no. 7, pp. 1056–1063, 2010.

[2] G. P. Garlet, "Critical reviews in oral biology & medicine: destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints," *Journal of Dental Research*, vol. 89, no. 12, pp. 1349–1363, 2010.

[3] C. M. Queiroz-Junior, M. F. Madeira, F. M. Coelho et al., "Experimental arthritis triggers periodontal disease in mice: involvement of TNF-$\alpha$, and the oral Microbiota," *Journal of Immunology*, vol. 187, no. 7, pp. 3821–3830, 2011.

[4] L. Zhao, Y. Zhou, Y. Xu, Y. Sun, L. Li, and W. Chen, "Effect of non-surgical periodontal therapy on the levels of Th17/Th1/Th2 cytokines and their transcription factors in Chinese chronic periodontitis patients," *Journal of Clinical Periodontology*, vol. 38, no. 6, pp. 509–516, 2011.

[5] P. J. Baker, "The role of immune responses in bone loss during periodontal disease," *Microbes and Infection*, vol. 2, no. 10, pp. 1181–1192, 2000.

[6] G. P. Garlet, W. Martins, B. R. Ferreira, C. M. Milanezi, and J. S. Silva, "Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease," *Journal of Periodontal Research*, vol. 38, no. 2, pp. 210–217, 2003.

[7] S. L. Gaffen and G. Hajishengallis, "A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17," *Journal of Dental Research*, vol. 87, no. 9, pp. 817–828, 2008.

[8] L. E. Harrington, R. D. Hatton, P. R. Mangan et al., "Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages," *Nature Immunology*, vol. 6, no. 11, pp. 1123–1132, 2005.

[9] C. Abraham and J. Cho, "Interleukin-23/Th17 pathways and inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 15, no. 7, pp. 1090–1100, 2009.

[10] J. C. López, "Immunology: targeting IL-17 in psoriasis," *Nature Medicine*, vol. 18, p. 671, 2012.

[11] S. Shahrara, Q. Huang, A. M. Mandelin, and R. M. Pope, "TH-17 cells in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 10, no. 4, article R93, 2008.

[12] R. B. Johnson, N. Wood, and F. G. Serio, "Interleukin-11 and IL-17 and the pathogenesis of periodontal disease," *Journal of Periodontology*, vol. 75, no. 1, pp. 37–43, 2004.

[13] S. R. Lester, J. L. Bain, R. B. Johnson, and F. G. Serio, "Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss," *Journal of Periodontology*, vol. 78, no. 8, pp. 1545–1550, 2007.

[14] K. Takahashi, T. Azuma, H. Motohira, D. F. Kinane, and S. Kitetsu, "The potential role of interleukin-17 in the immunopathology of periodontal disease," *Journal of Clinical Periodontology*, vol. 32, no. 4, pp. 369–374, 2005.

[15] R. Vernal, N. Dutzan, A. Chaparro, J. Puente, M. A. Valenzuela, and J. Gamonal, "Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis," *Journal of Clinical Periodontology*, vol. 32, no. 4, pp. 383–389, 2005.

[16] M. A. Eskan, R. Jotwani, T. Abe et al., "The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss," *Nature Immunology*, vol. 13, no. 5, pp. 465–473, 2012.

[17] H. Ohyama, N. Kato-Kogoe, A. Kuhara et al., "The involvement of IL-23 and the Th 17 pathway in periodontitis," *Journal of Dental Research*, vol. 88, no. 7, pp. 633–638, 2009.

[18] M. L. Laine, W. Crielaard, and B. G. Loos, "Genetic susceptibility to periodontitis," *Periodontology 2000*, vol. 58, no. 1, pp. 37–68, 2012.

[19] V. Pravica, C. Perrey, A. Stevens, J. H. Lee, and I. V. Hutchinson, "A single nucleotide polymorphism in the first intron of the human IFN-$\gamma$ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-$\gamma$ production," *Human Immunology*, vol. 61, no. 9, pp. 863–866, 2000.

[20] A. Paradowska-Gorycka, E. Wojtecka-Lukasik, J. Trefler, B. Wojciechowska, J. K. Lacki, and S. Maslinski, "Association between IL-17F gene polymorphisms and susceptibility to and severity of rheumatoid arthritis (RA)," *Scandinavian Journal of Immunology*, vol. 72, no. 2, pp. 134–141, 2010.

[21] G. B. N. Nordang, M. K. Viken, J. E. Hollis-moffatt et al., "Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand," *Rheumatology*, vol. 48, no. 4, pp. 367–370, 2009.

[22] T. Arisawa, T. Tahara, T. Shibata et al., "The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis," *Journal of Clinical Immunology*, vol. 28, no. 1, pp. 44–49, 2008.

[23] G. C. Armitage, "Development of a classification system for periodontal diseases and conditions," *Annals of Periodontology*, vol. 4, no. 1, pp. 1–6, 1999.

[24] G. C. Armitage, "Periodontal diagnoses and classification of periodontal diseases," *Periodontology 2000*, vol. 34, pp. 9–21, 2004.

[25] J. Alves-Silva, M. Da Silva Santos, P. E. M. Guimaraes et al., "The ancestry of Brazilian mtDNA lineages," *American Journal of Human Genetics*, vol. 67, no. 2, pp. 444–461, 2000.

[26] F. C. Parra, R. C. Amado, J. R. Lambertucci, J. Rocha, C. M. Antunes, and S. D. J. Pena, "Color and genomic ancestry in Brazilians," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 1, pp. 177–182, 2003.

[27] E. Newbrun, "Indices to measure gingival bleeding," *Journal of Periodontology*, vol. 67, no. 6, pp. 555–561, 1996.

[28] C. M. Queiroz-Junior, C. M. Pacheco, A. H. Fonseca et al., "Myeloperoxidase content is a marker of systemic inflammation in a chronic condition: the example given by the periodontal disease in rats," *Mediators of Inflammation*, vol. 2009, Article ID 760837, 2009.

[29] A. Beklen, M. Ainola, M. Hukkanen, C. Gürgan, T. Sorsa, and Y. T. Konttinen, "MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis," *Journal of Dental Research*, vol. 86, no. 4, pp. 347–351, 2007.

[30] T. Honda, Y. Aoki, N. Takahashi et al., "Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease," *Clinica Chimica Acta*, vol. 395, no. 1-2, pp. 137–141, 2008.

[31] J. J. Yu, M. J. Ruddy, G. C. Wong et al., "An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals," *Blood*, vol. 109, no. 9, pp. 3794–3802, 2007.

[32] H. A. Schenkein, T. E. Koertge, C. N. Brooks, R. Sabatini, D. E. Purkall, and J. G. Tew, "IL-17 in sera from patients with aggressive periodontitis," *Journal of Dental Research*, vol. 89, no. 9, pp. 943–947, 2010.

[33] N. Dutzan, J. Gamonal, A. Silva, M. Sanz, and R. Vernal, "Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-$\kappa$ B ligand, interleukin (IL) -17, IL-10 and transforming growth factor-$\beta$ during the progression of chronic periodontitis," *Journal of Clinical Periodontology*, vol. 36, no. 5, pp. 396–403, 2009.

[34] M. Hernandez, J. Gamonal, T. Tervahartiala et al., "Associations between matrix metalloproteinase-8 and-14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: a longitudinal study," *Journal of Periodontology*, vol. 81, no. 11, pp. 1644–1652, 2010.

[35] L. Konopka, A. Pietrzak, and E. Brzeziñska-Błaszczyk, "Effect of scaling and root planing on interleukin-1â, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients," *Journal Periodontal Research*, vol. 47, no. 6, pp. 681–688, 2012.

[36] D. F. Kinane and T. C. Hart, "Genes and gene polymorphisms associated with periodontal disease," *Critical Reviews in Oral Biology and Medicine*, vol. 14, no. 6, pp. 430–449, 2003.

[37] R. M. Scarel-Caminaga, P. C. Trevilatto, A. P. Souza, R. B. Brito, and S. R. P. Line, "Investigation of IL4 gene polymorphism in individuals with different levels of chronic periodontitis in a Brazilian population," *Journal of Clinical Periodontology*, vol. 30, no. 4, pp. 341–345, 2003.

[38] P. C. Trevilatto, R. M. Scarel-Caminaga, R. B. de Brito Jr. et al., "Polymorphism at position _174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population," *Journal of Clinical Periodontology*, vol. 30, no. 1, pp. 438–442, 2003.

[39] S. M. Jaradat, K. T. Ababneh, S. A. Jaradat et al., "Association of interleukin-10 gene promoter polymorphisms with chronic and aggressive periodontitis," *Oral Disease*, vol. 18, no. 3, pp. 271–279, 2012.

[40] N. Babel, G. Cherepnev, D. Babel et al., "Analysis of tumor necrosis factor-$\alpha$, transforming growth factor-$\beta$, interleukin-10, IL-6, and interferon-$\gamma$ gene polymorphisms in patients with chronic periodontitis," *Journal of Periodontology*, vol. 77, no. 12, pp. 1978–1983, 2006.

[41] G. K. Nikolopoulos, N. L. Dimou, S. J. Hamodrakas, and P. G. Bagos, "Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls," *Journal of Clinical Periodontology*, vol. 35, no. 9, pp. 754–767, 2008.

[42] N. Oda, P. B. Canelos, D. M. Essayan, B. A. Plunkett, A. C. Myers, and S. K. Huang, "Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 1, pp. 12–18, 2005.

[43] E. S. Kim, S. W. Kim, C. M. Moon et al., "Interactions between IL17A, IL23R, and STAT4 polymorphisms confer susceptibility to intestinal Behcet's disease in Korean population," *Life Sciences*, vol. 90, no. 19-20, pp. 740–746, 2012.

[44] L. Wang, Y. Jiang, Y. Zhang et al., "Association analysis of IL-17A and IL-17F polymorphisms in Chinese han women with breast cancer," *PLoS ONE*, vol. 7, no. 3, Article ID e34400, 2012.

[45] X. Wu, Z. Zeng, B. Chen et al., "Association between polymorphisms in interleukin-17A and interleukin-17F genes and risks of gastric cancer," *International Journal of Cancer*, vol. 127, no. 1, pp. 86–92, 2010.

[46] S. Liang, K. B. Hosur, H. Domon, and G. Hajishengallis, "Periodontal inflammation and bone loss in aged mice," *Journal of Periodontal Research*, vol. 45, no. 4, pp. 574–578, 2010.

[47] J. P. Allam, Y. Duan, F. Heinemann et al., "IL-23 producing CD681 macrophage-like cells predominate within a IL-17 polarized infiltrate in chronic periodontitis lesions," *Journal of Clinical Periodontology*, vol. 38, no. 1, pp. 879–886, 2011.

[48] W. Ouyang, J. K. Kolls, and Y. Zheng, "The biological functions of T helper 17 cell effector cytokines in inflammation," *Immunity*, vol. 28, no. 4, pp. 454–467, 2008.

[49] G. Page and P. Miossec, "RANK and RANKL expression as markers of dendritic cell-T cell interactions in paired samples of rheumatoid synovium and lymph nodes," *Arthritis and Rheumatism*, vol. 52, no. 8, pp. 2307–2312, 2005.

[50] K. Sato, A. Suematsu, K. Okamoto et al., "Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction," *Journal of Experimental Medicine*, vol. 203, no. 12, pp. 2673–2682, 2006.

[51] F. Fossiez, O. Djossou, P. Chomarat et al., "T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines," *Journal of Experimental Medicine*, vol. 183, no. 6, pp. 2593–2603, 1996.

[52] Y. Hosokawa, I. Hosokawa, K. Ozaki, H. Nakae, and T. Matsuo, "Cytokines differentially regulate CXCL10 production by interferon—stimulated or tumor necrosis factor—stimulated human gingival fibroblasts," *Journal of Periodontal Research*, vol. 44, no. 2, pp. 225–231, 2009.

[53] R. Mahononda, P. Jitprasertwong, N. Sa-Ard-Iam et al., "Effects of IL-17 on human gingival fibroblasts," *Journal of Dental Research*, vol. 87, no. 3, pp. 267–272, 2008.

[54] P. Miossec, T. Korn, and V. K. Kuchroo, "Interleukin-17 and type 17 helper T cells," *The New England Journal of Medicine*, vol. 361, no. 9, pp. 848–898, 2009.

# 3.2 Artigo II

# Polymorphisms of *IL-10* and *TNFA* genes and inflammation status in chronic periodontitis

Jôice Dias Corrêa[1], Daniele Sirineu Pereira[2], Mila Fernandes Moreira Madeira[3], Danielle da Glória de Souza[3], Mauro Martins Teixeira[4], Antônio Lúcio Teixeira[5], José Eustáquio Costa[1], Celso Martins Queiroz-Junior[1], Tarcília Aparecida da Silva[1*]

[1]Department of Oral Surgery and Pathology, School of Dentistry

[2]Department of Physical Therapy, School of Physical Education, Physical Therapy and Occupational Therapy

[3]Department of Microbiology, Biological Sciences Institute,

[4]Department of Biochemistry and Immunology, Biological Sciences Institute,

[5]Department of Clinical Medicine, Faculty of Medicine,

Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

**Keywords:** periodontal disease, genetic polymorphism, interleukin-10, TNF-α

**Running title:** *IL-10* and *TNF* polymorphisms in periodontitis

**Conflict of Interest and Source of Funding Statement:** The authors declare that they have no conflict of interests. This study was supported by CAPES, CNPq and FAPEMIG.

[*] **Corresponding author**: Tarcília Aparecida da Silva, Ph.D. Mailing address: Departamento de Patologia e Cirurgia Odontológica, Faculdade de Odontologia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, CEP 31.270-901, Belo Horizonte, Minas Gerais, Brazil. Phone: 55 31 3409-2478 (voice); 55 31 3409-2430 (Fax). E-mail: tarcilia@ufmg.br

## ABSTRACT

**Aim:** Polymorphisms in genes that regulate the immune response may have an impact on the susceptibility to and progression of chronic periodontitis (CP). In this study, we analyzed the interleukin-10 (IL-10) rs1800896 and tumor necrosis factor-alpha (TNF-α) rs1800629 gene single nucleotide polymorphisms and their association with the periodontal status of subjects with CP and levels of gingival inflammatory mediators.

**Material and Methods:** Genomic DNA was obtained from 30 CP patients and 30 periodontally healthy controls (HC). Genotyping of *TNFA* (rs1800629) and *IL10* (rs1800896) was performed by the TaqMan PCR Method. Periodontal tissues were collected and processed for ELISA, myeloperoxidase (MPO) and microscopic analysis.

**Results**: The frequencies of *IL-10* genotypes in the CP group were significantly different from HC. The AG genotype was correlated with worse clinical parameters (bleeding on probing) and high MPO activity, while the GG genotype showed increased levels of IL-10 and decreased levels of CXCL10 in gingival tissues. In regard to *TNFA* polymorphism no correlation with the periodontal status or inflammatory markers has been found.

**Conclusions**: These data suggest that the *IL-10* polymorphism contributes to worse periodontal status in periodontal disease. On the other hand, *TNFA* polymorphism does not seem to affect periodontitis.

## INTRODUCTION

Periodontal health can be described as “a dynamic state where the activity of pro-inflammatory/antimicrobial cytokines to control infection is optimally balanced by anti-inflammatory mechanisms to prevent unwarranted inflammation” (Gaffen & Hajishengallis, 2008). In periodontitis, the major factors regulating the infection-induced destruction of teeth-supporting tissues are the inflammatory mediators released by host cells; these include a complex equilibrium of pro- and anti-inflammatory cytokines (Garlet et al., 2010). In susceptible hosts, an imbalance of the inflammatory response occurs, resulting in excessive production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, and the subsequent loss of periodontal attachment (Gemmel et al., 1997). On the other hand, controlling mechanisms are also important in this process, including the production of the anti-inflammatory cytokine interleukin-10 (IL-10) to prevent excessive tissue damage (Ouyang et al., 2011).

Both IL-10 and TNF-α are known for their central and opposing roles in antibacterial immune responses. Interleukin-10 may be critical in controlling the balance between T helper 1 and T helper 2 cells in chronic periodontitis, whereby an excess of interleukin-10 may shift the balance in favor of a T helper 2 response and chronic disease, whereas a deficiency may lead to increased cytokine production and tissue destruction in periodontitis. In fact, IL-10 is associated with chronic lesions in periodontal disease, and after the treatment, its levels in gingival crevicular fluid tend to increase, indicating that this cytokine may be associated with lower disease severity (Cutler et al., 2000; Garlet et al., 2004; 2006; Dutzan et al., 2009). On the other hand, TNF-α has received a considerable amount of attention because of its effects on bone destruction (DuFour et al., 1993, Manolagas, 1995, Offenbacher, 1996). Accordingly, numerous reports have described increased levels of TNF-α in crevicular fluid and periodontal tissues of patients with periodontitis (Graves & Cochran, 2003; Garlet et al., 2004; Graves, 2008). Therefore, it is seems reasonable to assume that differences in the levels of these cyto-

kines can affect the progression of periodontal disease. In this regard, there is considerable knowledge that the presence of functional polymorphisms in cytokine genes affects their expression and thus may have a role in the regulation of inflammatory response and resistance or susceptibility to infections (Kinane et al., 2003).

The gene encoding TNF-α has been mapped to chromosome 6 and a polymorphism in the locus –308, causing a substitution from the guanine (G) to adenine (A), leads to a higher production of TNF-α after stimulation with bacterial lipopolysaccharide (Wilson et al., 1997). The gene encoding IL-10 is located on the chromosome 1. The *IL-10* promoter region is highly polymorphic, and three SNPs at positions _1087 (A > G), _592 (C > T), and _819 (C > A) in the promoter region have been identified (Turner et al., 1997). These *IL-10* gene polymorphisms could be detrimental to host tissues and could be linked to periodontal disease susceptibility because *IL-10* SNPs have been associated with decreased IL-10 production (Turner et al., 1997). In fact some studies have tried to link the *IL-10* and *TNFA* polymorphisms and periodontal disease, but the results are conflicting (Berglundh et al., 2002; Lin et al., 2003; Moreira et al., 2009; Costa et al., 2010; Zhong et al., 2012).

Therefore, considering the importance of TNF-α and IL-10 in the pathogenesis of periodontitis, and the controversial results in the current literature about the presence of polymorphisms in *TNFA* and *IL10* genes and its relationship with periodontal disease, the current study aimed to evaluate the occurrence of the *IL-10* (-1087) and *TNFA* (-308) gene polymorphisms and the phenotype alteration in a sample of Brazilian patients with chronic periodontitis.

## MATERIAL AND METHODS

### Subjects and sample collection

Thirty patients with CP who attended at the Periodontal Clinic, School of Dentistry, at Universidade Federal de Minas Gerais (UFMG, Brazil) were enrolled in this research and the gingival tissue samples were obtained from periodontal tissues resected during periodontal surgery. The inclusion criteria were: patients with a previous history of CP, diagnosed according to previously described criteria, as follows: more than one tooth with probing depth higher than 5 mm, more than two sites with clinical attachment loss deeper than 6 mm and lesions distributed in more than two teeth in each quadrant (Armitage, 1999). Exclusion criteria were: use of antibiotic, anti-inflammatory and/or immunosuppressive medications in the preceding 6 months and any systemic diseases (i.e., immunologic and autoimmune disorders, diabetes mellitus). The control group (HC) comprised 30 periodontally healthy patients, age- and gender-matched to the CP group.

Periodontal examination was performed in both groups of patients, CP and HC, to determine probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP). The BOP was considered positive if bleeding occurred within 30 seconds after probing (Newbrun, 1996). Measurements were performed full-mouth at 6 sites per tooth (mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual and distolingual). At the time of the examination, a peripheral blood sample was taken from each patient and processed for polymorphism determination.

Written informed consent was obtained from all patients. This study protocol was approved by the local Institutional Ethics Committee (324/08).

**Inflammatory infiltrate evaluation**

Periodontal tissue samples were fixed in 10% buffered formalin, embedded in paraffin wax and cut longitudinally (3 µm). The sections were deparaffinized, rehydrated, and stained with H&E for evaluation of the inflammatory infiltration. Inflammatory cells were counted in four fields in two independent sections, using light microscope (Axioskop 40 ZEISS; Carl Zeiss, Gottingen, Germany) at 400x magnification. Data were expressed as total of inflammatory cells/field.

**ELISA**

The concentrations of the cytokines IL-10 and tumor necrosis factor (TNF)-α, and the chemokine CXCL10 were measured in gingival tissues by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (R&D Systems, Minneapolis, MN, USA).

The assay was performed according to the manufacturer´s instructions. The data were determined using a standard curve prepared for each assay and expressed as picograms of cytokine/chemokine per 100 mg of tissue.

**Myeloperoxidase**

Gingival tissue samples were also used for determination of myeloperoxidase (MPO) activity, a neutrophil enzyme marker, as described earlier (Queiroz-Junior et al., 2009). The MPO activity in homogenized gingival tissues was evaluated by enzymatic reaction, measured by absorbance at 450 nm. The MPO content was expressed as relative units calculated from standard curves based on MPO activities from 5% casein peritoneal-induced neutrophils assayed in parallel.

**DNA isolation and genotyping analysis**

Total genomic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit (Quiagen, Valencia, CA, USA) according to the manufacturer instructions. Quality, integrity and quantity of DNA were analyzed by Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

TaqMan genotyping assays were obtained from Applied Biosystems, Inc. (Foster City, CA, USA). The assay identification code for each respective SNP is *IL-10* (rs1800896) and *TNFA* (rs1800629). All amplifications were carried out in an ABI 7900H thermal cycler (Applied Biosystems, Foster City, CA, USA) using TaqMan Genotyping Master Mix and following the manufacturer's recommended amplification conditions.

**Data analysis**

Data were expressed as mean ± standard deviation (SD). Chi-square test analysis was used to test for deviation of genotype frequencies from Hardy-Weinberg equilibrium. The levels of cytokines in periodontal tissues and the frequency of gene polymorphisms were compared by the Mann Whitney or Kruskal-Wallis and chi-square test. $p$ values $< 0.05$ were considered statistically significant. All data were analyzed using SPSS 17 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Genotypic frequency analysis in the HC and CP groups

The sample included in the current study was composed by age- and gender-matched groups. The frequency of smoker subjects was not statistically significant and these patients were excluded from the study.

The clinical features PD, CAL and BOP were significantly higher in the CP than in the HC group ($p < 0.0001$) (Table 1).The levels of inflammatory mediators IL-10 e TNF-α in periodontal tissues were greater in CP patients than controls (Figure 1A and 1B). Moreover, the MPO activity and the inflammatory infiltrate in the gingival tissue, characterized by polymorphonuclear and mononuclear leukocytes, were significantly higher in the CP than in the HC group (Figure 1C, D).

Following the clinical investigation, the frequencies of polymorphisms (*IL-10* and *TNFA*) were investigated in blood samples of HC and CP subjects (Table 2). The frequency of these genotypes agreed with the Hardy-Weinberg equilibrium ($p > 0.05$). The *IL-10* AA genotypes of the group CP (AA 50.05%) were significantly different from the frequencies observed in the group HC (AA 17.40%), ($p = 0.01$) (Table 2). In contrast, the distribution of the *TNFA* polymorphisms was similar between the groups (Table 2).

**Association among polymorphisms and clinical periodontal parameters**

We also investigated whether some of these polymorphisms were associated with worse clinical periodontal parameters. As shown in Table 3, the intra-group comparison of the *IL-10* genotypes indicated that BOP was significantly higher in AG subjects than in patients with the GG genotype (table 3). Concerning *TNFA* polymorphisms, no differences in clinical parameters were found when comparing the genotypes.

***IL-10* polymorphisms are related with inflammatory markers in periodontium**

The association among *IL-10* gene polymorphisms and the presence of inflammatory mediators in periodontal tissues was also investigated. As shown in Figure 2, the levels of IL-10 in periodontal tissues from cases were increased in GG genotype when comparing the *IL-10* genotypes to each other, while the levels of CXCL10 were decreased (Figure 2A, B). The MPO activity was higher in the AG genotype (Figure 2C), but the whole inflammatory infiltrate was equally distributed among the evaluated groups (AA: 103.5 ± 20.68; AG: 102.5 ± 2.46; GG: 82.5 ± 18.31 inflammatory cells/field; $p= 0.15$).

***TNFA* polymorphisms are not associated with inflammatory parameters in periodontal tissues**

The levels of TNF-α and the MPO activity in gingival tissues did not differ when comparing the *TNFA* genotypes (Figure 3). There were no differences in the inflammatory infiltrate among the polymorphisms groups (AG: 85.3 ± 32.16; GG: 91.75 ± 17.18 inflammatory cells/field; $p=0.71$).

**Discussion**

The present study has shown a relationship between *IL-10* genotype and periodontal disease in a sample of Brazilian patients. The frequency of the *IL-10* genotype AA was increased in subjects with chronic periodontitis when compared with healthy controls.

In addition, our results showed that subjects with the *IL-10* genotype GG had increased expression of IL-10 and decreased CXCL10 expression in periodontal tissues, and also exhibited a tendency of better periodontal condition in relation to the AG genotype.

In agreement with our results, some studies have suggested that the *IL-10* gene polymorphisms may be associated with susceptibility to periodontitis (Berglundh et al., 2002; Scarel-Caminaga et al., 2004; Sumer et al., 2007; Cullinan et al., 2008; Atanasovska-Stojanovska et al., 2012), whereas other studies have failed to demonstrate such association (Kinane et al., 1999; Yamazaki et al., 2001; Gonzales et al., 2002; Brett et al., 2005; Babel et al., 2006; Tervonen et al., 2007; Moreira et al., 2009; Gurol et al., 2011). The phenotype resulting from *IL-10* polymorphism has already been demonstrated (Turner et al., 1997). In this case, a G to A transition at _1087 is related to regulation of IL-10 production in which the allele G is associated with a high production, while the allele A corresponds to a decreased IL-10 production (Turner et al., 1997). It may therefore be anticipated that a homozygous GG _1087 genotype indicates considerably enhanced IL-10 production, a heterozygous AG genotype presents moderate production and that, finally, a homozygous AA genotype reveals suppressed IL-10 production (Atanasovska-Stojanovska et al., 2012), confirming our results which show the GG genotype as a higher producer of IL-10.

Among the several functions of IL-10 during infectious conditions is its influence in the expression of immune mediators (Paul et al., 2012). IL-10 can inhibit the release of pro-inflammatory cytokines from monocytes/macrophages and therefore inhibits the lipopolysaccharide- and IFN-γ-induced secretion of TNF-α, IL-1β, IL-6, CXCL8,

granulocyte-colony-stimulating factor and granulocyte–macrophage colony-stimulating factor (Fiorentino et al., 1991). Nevertheless, in the current study, we could not find decreased levels of the evaluated cytokines, except for the chemokine CXCL10, in the same subjects with increased levels of IL-10. These findings suggest that, despite the increased production of IL-10 associated with the genotype GG in the evaluated *IL-10* polymorphism, there was an active inflammatory scenario characterized by the release of a wide range of inflammatory cytokines, without a clear clinical periodontal protection. On the other hand, although the allele A appeared to be associated with a worse prognosis, mainly given the higher MPO activity and bleeding on probing found in patients with the AG genotype, the current data are not conclusive, since most of the control patients also had the AG genotype of *IL10*. In fact, previous studies have found that the allele A may be correlated with protection to periodontal disease (Atanasovska-Stojanovska et al., 2012; Berglundh et al., 2002). In contrast, another study by Yamazaki et al., (2001) in a Japanese population, demonstrated that none of the genotypes could be linked to subjects with periodontitis, and the occurrence of the allele G at the position _1087 in such patients occurred in relative low terms. These varying results could be attributed to several factors related to the definition of disease, population heterogeneity, environmental and demographic confounding risk factors.

Regarding the *TNFA* polymorphism, in the current study, there were no differences in the genotype distribution between periodontitis patients and control subjects. Some studies have shown that a guanine (G) to adenine (A) transition at position -308 of the *TNFA* promoter affects a consensus sequence for a binding site of the transcription factor AP-2 (Abraham et al., 1999). Carriage of the rare -308A allele is associated with significantly greater TNF-α production and transcription (Abraham et al., 1999). In addition, the allele A has been associated with increased risk for various non-related infectious and inflammatory disorders (Wilson et al., 1995), including periodontitis (Lin et al., 2003). However, our results are in

agreement with several studies that have failed to corroborate the association between this polymorphism and increased risk for periodontal diseases (Craandijk et al., 2002; Fassmann et al., 2003; Folwaczny et al., 2004; Donati et al., 2005; Sakellari et al., 2006; Menezes & Colombo, 2007; Moreira et al., 2009; Costa et al., 2010). In contrast to these studies, Galbraith et al., (1999) and Ricci et al., (2011), found an association between *TNFA* polymorphism and periodontal disease, and another conflicting study found a higher frequency of homozygous (G/G) genotype in individuals with periodontitis when compared to controls in Egyptians (Settin et al., 2006). In view of these data, it has been reported that not only SNP, but specific combinations of functional polymorphisms in different genes may significantly alter the individual risk to development of a phenotype in a disease (Walker et al., 2000), hypothesis that could explain all these differences in the literature.

In conclusion, the *IL-10* gene polymorphism seems to be related with CP; on the other hand, the association between *TNFA* polymorphism and chronic periodontal disease does not seem to be significant. Future studies with a large cohort of patients and additional examinations concerning the influence of each genotype in the inflammatory response in subjects with periodontitis could help to strengthen and understand the role of genetic polymorphisms in periodontal disease.

REFERENCES

ABRAHAM L. J.; KROEGER, K. M. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol*. 66(4):562-6, 1999.

ARMITAGE, G.C. Development of a classification system for periodontal disease and conditions. *Ann Periodontol*, Chicago, 4(1): 1-6, 1999.

ATANASOVSKA-STOJANOVSKA, A. et al. IL10 -1087, IL10 -819 and IL10 -592 polymorphisms are associated with chronic periodontitis in a Macedonian population. *Hum Immunol*. 73(7):753-8, 2012.

BABEL, N. et al. Analysis of tumor necrosis factor-alpha, transforming growth factor beta, interleukin- 10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J Periodontol.* 77:1978–1983, 2006.

BERGLUNDH, T. Association of the -1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol*. 30:249–254, 2003.

BRITO, L. C. et al. T-lymphocyte and cytokine expression in human inflammatory periapical lesions. *J Endod*. 38(4):481-5, 2012.

BRETT, P. M. et al. Functional gene polymorphisms in aggressive and chronic periodontitis. *J Dent Res.* 84:1149–1153, 2005.

COSTA, A. M. et al. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. *Gerodontology.* 27(1):70-5. 2010.

CRAANDIJK, J. et al. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *J Clin Periodontol*. 29(1):28-34, 2002.

CULLINAN, M. P. et al. Progression of periodontal disease and interleukin-10 gene polymorphism. *J Periodontal Res*. 43(3):328-33, 2008.

CUTLER, C. W. Clinical benefits of oral irrigation for periodontitis are related to reduction of pro-inflammatory cytokine levels and plaque. *J Clin Periodontol*. 27(2):134-43, 2000.

DONATI, M. et al. Association of the -159 CD14 gene polymorphism and lack of association of the -308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol*. 32(5):474-9, 2005.

DUFOUR, A., et al. Regulation of differentiation of gingival fibroblasts and periodontal ligament cells by rhIL-1*b* and rhTNF-*a*. *Journal of Periodontal Research* 28, 566–568, 1993.

DUTZAN, N. et al. Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-*κ* B ligand, interleukin (IL) -17, IL-10 and transforming growth factor-*β* during the progression of chronic periodontitis. *Journal of Clinical Periodontology*. 36(5): 396–403, 2009.

FASSMANN, A. et al. Polymorphisms in the +252(A/G) lymphotoxin-alpha and the -308(A/G) tumor necrosis factor-alpha genes and susceptibility to chronic periodontitis in a Czech population. *J Periodontal Res*. 38(4):394-9, 2003.

FIORENTINO, D. F. et al. IL-10 inhibits cytokine production by activated macrophages. *J Immunol*. 147(11):3815-22, 1991.

FOLWACZNY, M. et al. Lack of association between the TNF alpha G -308 A promoter polymorphism and periodontal disease. *J Clin Periodontol*. 31(11):449-53, 2004.

GAFFEN, S. L.; HAJISHENGALLIS, G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *J Dent Res*. 87(9):817-28, 2008.

GALBRAITH, G. M, et al. Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype. *J Periodontol.* 69: 428–433, 1998.

GARLET, G. P. *et al*. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *Journal of Clinical Periodontology*, 31 (8):671-679, 2004.

GARLET, G. P. *et al.* Cytokine pattern determines the progression of experimental periodontal disease induced by *Actinobacillus actinomycetemcomitans* through the modulation of MMPs, RANKL and their physiological inhibitors. *Oral Microbiol. Immunol.* 21:12-20, 2006.

GARLET, G. P. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. *Journal of Dental Research.* 89: 1349-1363, 2010.

GEMMELL, E.; MARSHALL, R. I.; SEYMOUR, G. J. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol 2000.* 14:112–143, 1997.

GONZALES, J. R. et al. Analysis of genetic polymorphisms at the interleukin-10 loci in aggressive and chronic periodontitis. *J Clin Periodontol.* 29:816–822, 2002.

GRAVES, D.T. & COCHRAN, D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology.* 74( 3);391-401, 2003.

GRAVES, D. T. Cytokines that promote periodontal tissue destruction. *Journal of Periodontology*. 79: Suppl 8; 1585-1591, 2008.

GUROL, C. et al. A comparative study of the role of cytokine polymorphisms interleukin-10 and tumor necrosis factor alpha in susceptibility to implant failure and chronic periodontitis. *Int J Oral Maxillofac Implants*. 26(5):955-60, 2011.

KINANE, D. F. et al. Analysis of genetic polymorphisms at the interleukin-10 and tumour necrosis factor loci in early-onset periodontitis. *J Periodont Res*. 34:379–386, 1999.

KINANE, D. F.; HART, T. C. Genes and gene polymorphisms associated with periodontal disease. *Crit Rev Oral Biol Med*.14(6):430-49, 2003.

LIN, L.; PAN, Y. P.; YIN, L. Y. Study on the correlation of cytokine gene polymorphism with chronic periodontitis. *Shanghai Kou Qiang Yi Xue*. 12(6):456-9, 2003.

MANOLAGAS, S. C. Role of cytokines in bone resorption. *Bone* 17, 63S–67S, 1995.

MENEZES, N. G.; COLOMBO, A. P. Lack of association between the TNF-alpha -308 (G/A) genetic polymorphism and periodontal disease in Brazilians. *Braz Oral Res.* 22(4):322-7, 2008.

MOORE, K. W. et al. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol*. 19:683–765, 2001.

MOREIRA, P. R., et al. TNFA and IL10 gene polymorphisms are not associated with periodontitis in Brazilians. *Open Dent J.* 7; 3:184-90, 2009.

NEWBRUN, E. Indices to measure gingival bleeding. *Journal of Periodontology.* 67: 555-561, 1996.

OFFENBACHER, S. Periodontal diseases: pathogenesis. *Annals of Periodontology* 1,821–878, 1993.

OUYANG, W. et al. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol*. 29:71-109, 2011.

PAUL, G; KHARE, V; GASCHE, C. Inflamed gut mucosa: downstream of interleukin-10. *Eur J Clin Invest*. 42(1):95-109, 2012.

QUEIROZ-JUNIOR, C. M. et al. Myeloperoxidase content is a marker of systemic inflammation in a chronic condition: the example given by the periodontal disease in rats. *Mediators of Inflammation*. 760837, 2009.

RICCI, M. et al., Association between genetic risk score and periodontitis onset and progression: A pilot study. *archives of oral biology*. 56(12):1499 – 1505, 2011.

SAKELLARI, D. et al. No correlation of five gene polymorphisms with periodontal conditions in a Greek population. *J Clin Periodontol*. 33(11):765-70, 2006.

SCAREL-CAMINAGA, R. M. et al. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. *J Clin Periodontol*. 31:443–448, 2004.

SETTIN, A. et al. Gene polymorphisms of TNF-□-308(G/A), IL-10-1087(G/A), IL6-174(G/C) and IL1Ra(VNTR) in Egyptian cases with adult and early onset periodontitis. *Internet J Den Sci*. 4(1), 2006.

STASHENKO, P. et al. Tissue levels of bone resorptive cytokines in periodontal disease. *Journal of Periodontology*. 62, 504–509, 1991.

SUMER, A. P. et al. Association of interleukin-10 gene polymorphisms with severe generalized chronic periodontitis. *J Periodontol*. 78(3):493-7, 2007.

TERVONEN, T. et al. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. *J Clin Periodontol*. 34(5):377-83, 2007.

TURNER, D. M., et al. An investigation of polymorphism in the interleukin-10 gene promoter. *European Journal of Immunogenetics*. 24, 1–8, 1997.

WALKER, S. J. et al. Genetic polymorphisms of the IL-1α and Il-1β genes in African-American LJP patients and an African-American control population. *J. Periodontol.* 71: 723-8, 2000.

WILSON, A. G.; DI GIOVINE, F. S; DUFF, G. W. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *J Inflamm*. 45(1):1-12, 1995.

YAMAZAKI, K. et al. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *J Clin Periodontol*. 28(9):828-32, 2001.

ZHONG, Q. et al. Interleukin-10 gene polymorphisms and chronic/aggressive periodontitis susceptibility: a meta-analysis based on 14 case-control studies. *Cytokine.* 60(1):47-54, 2012.

*Table 1.* Demographic and clinical features of the studied subjects

| | **HC (N= 28)** | **CP (N= 29)** | ***p-value*** |
|---|---|---|---|
| **Age (SD)** | 41.21 (8.4) | 44.4 (8.5) | 0.25 |
| **Gender (% F)** | 56.5 | 51.20 | 0.63 |
| **PD (SD)** | 2.60 (0.7) | 4.46 (0.9)* | < 0.0001 |
| **CAL (SD)** | 2.06 (0.9) | 5.07 (0.8)* | < 0.0001 |
| **BOP (SD)** | 5.7 (0.6) | 31.6 (2.2)* | <0.0001 |

HC: Healthy controls, CP: Chronic Periodontitis

SD: standard deviation, PD: probing depth, CAL: Clinical attachment loss, BOP: Bleeding on probing (% of sites)

* Significantly higher than control ($X^2$ test or Mann-Whitney)

*Table 2.* Genotypes of *IL-10* and *TNFA* polymorphisms in patients with chronic periodontitis (CP) and healthy controls (HC)

| **Genotype** | **HC (%)** | **CP (%)** | ***p-value*** | **OR (95% CI)** |
|---|---|---|---|---|
| ***IL-10* rs1800896** | | | | |
| AA | 17.02 | 53.15* | *< 0.001* | 0.2 (0.12 - 0.40) |
| AG | 78.57 | 42.85 | *0.76* | 1.2 (0.15 – 7.10) |
| GG | 4.41 | 4.0 | *0.91* | 1 |
| ***TNFA* rs1800629** | | | | |
| GG | 71.42 | 75.86 | *0.97* | 2.5 (0.78 - 7.50) |
| AG | 28.57 | 24.13 | *0.71* | 1 |
| AA | 0 | 0 | | |

OR: Odds Ratio, CI: Confidence interval

* Significantly different from control $p < 0.05$ ($X^2$ test)

*Table 3*. Association between *IL-10* and *TNFA* polymorphisms and clinicopathological features of chronic periodontitis

| **Genotype** | **PD (mm)** | ***p-value*** | **CAL (mm)** | ***p-value*** | **BOP (%)** | ***p-value*** |
|---|---|---|---|---|---|---|
| **IL-10 rs1800896** | | | | | | |
| AA | 4.54 | *0.168* | 5.04 | *0.096* | 24.83 | *0.180* |
| AG | 4.92 | *0.164* | 4.55 | *0.400* | 39.45* | *0.037* |
| GG | 4.00 | | 5.00 | | 16.00 | |
| ***TNFA* rs1800629** | | | | | | |
| AG | 4.20 | *0.107* | 5.40 | *0.590* | 39.45 | *0.058* |
| GG | 4.72 | | 5.25 | | 24.10 | |

* Significantly different from GG ($p < 0.05$, Kruskal-Wallis or Mann Whitney test)

Figure 1.

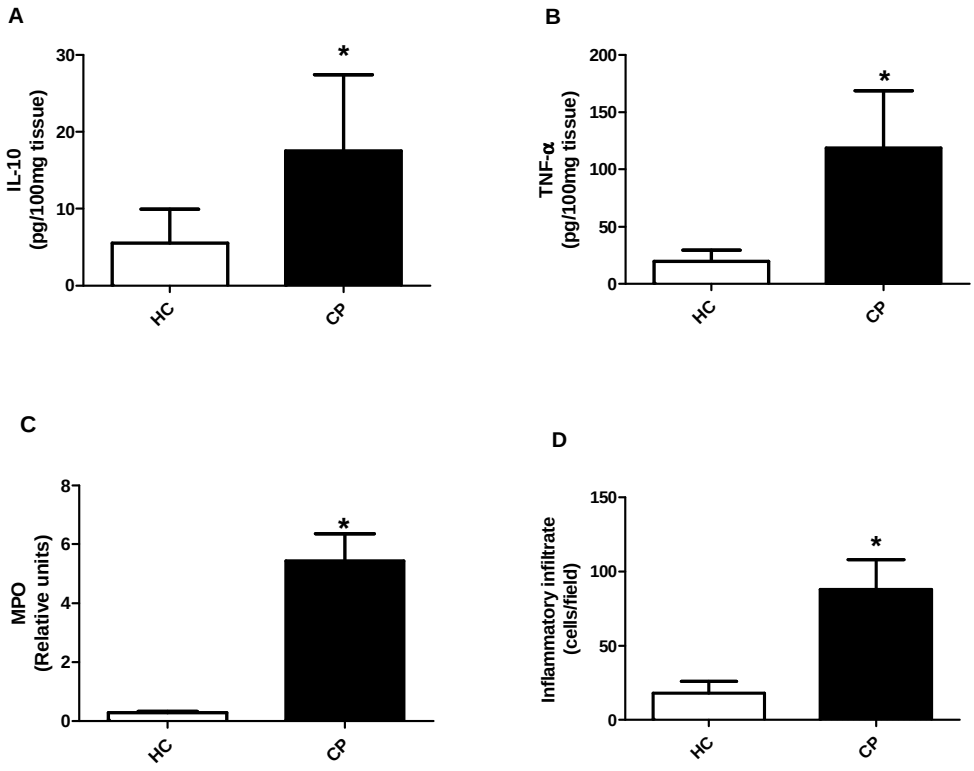


*Fig. 1.* Levels of (a) IL-10, (b) TNF-α, (c) MPO activity and (d) number of inflammatory cells in the gingival tissue from CP and HC subjects. *Statistically significant difference at $p < 0.05$. HC: Healthy Control; CP: Chronic Periodontitis; MPO: Myeloperoxidase

Figure 2.

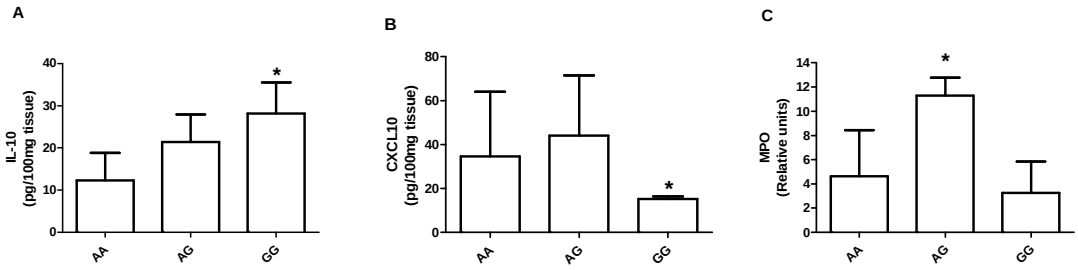


*Fig. 2.* Levels of (a) IL-10, (b) CXCL10 and (c) MPO (myeloperoxidase) in periodontal tissues of patients with chronic periodontitis according to each *IL-10* genotype. * Statistically significant different in comparison to the other genotypes ($p < 0.05$).

Figure 3.

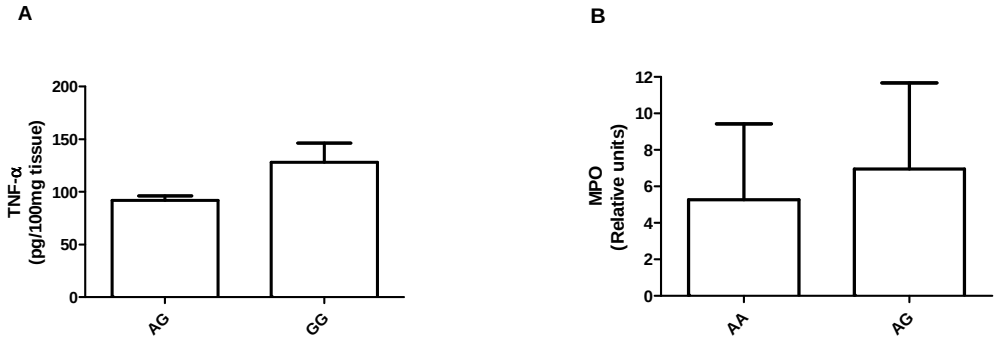


*Fig. 3.* Levels of inflammatory mediators in chronic periodontitis patients according to each *TNFA* genotype. (a) Levels of TNF-α in gingival tissues and (b) MPO (myeloperoxidase) activity in gingival tissue samples.

# 4 CONSIDERAÇÕES FINAIS

A doença periodontal é decorrente da infecção dos tecidos de suporte dos dentes, sendo caracterizada pela inflamação e perda desses tecidos (ARMITAGE, 1999). Embora o biofilme microbiano seja considerado a causa primária das DPs, é importante destacar o papel do sistema imune no desenvolvimento da doença.

A resposta imunológica desencadeada pelos periodontopatógenos, em conjunto com a suscetibilidade do paciente, determinam o desfecho da doença (KINANE *et al*., 2005; OFFENBACHER *et al*., 2008). Neste contexto, a presença de alterações genéticas, que determinam a expressão dos genes do sistema imune, pode interferir na patogênese da DP.

De acordo com Loos, John e Laine (2005) vários estudos tentaram determinar quais genes envolvidos na resposta imune seriam candidatos a fatores de risco para o desenvolvimento da DP. No presente trabalho, verificamos a presença e a influência de polimorfismos genéticos das citocinas pró-inflamatórias IL-17A, IL-17F e TNF-α e também da citocina anti-inflamatória IL-10 na DP.

A presença do alelo A de *IL-17A* foi relacionada a uma maior susceptibilidade para periodontite crônica em nosso estudo. Esses achados estão de acordo com estudos de outros grupos que demonstraram uma associação entre polimorfismos de *IL-17A* e outras desordens inflamatórias como artrite reumatóide (NORDANG *et al.*, 2009), doença de Behçet (KIM *et al.*, 2012), colite ulcerativa (ARISAWA *et al.*, 2008), câncer gástrico (WU *et al*., 2010) e câncer de mama (WANG *et al*., 2012). Ao contrário, o estudo de Saraiva *et al*., (2012) encontrou o alelo A como fator protetor para doença periodontal. Essa diferença nos resultados pode ser atribuída à metodologia diferente da detecção do polimorfismo, tamanho da amostra ou até mesmo ao não pareamento entre doentes e controles.

Em nosso estudo, além do alelo A estar mais presente nos pacientes com periodontite, este alelo demonstrou estar relacionado a uma maior gravidade da doença, indicada pelos parâmetros clínicos (profundidade de sondagem maior nos portadores do alelo A), maior expressão de mediadores inflamatórios no soro e tecidos periodontais (IL-17A e CXCL8/IL-8) e aumento do recrutamento de neutrófilos nos sítios periodontais (indicada pela maior atividade de MPO). Apesar de não podermos comprovar os mecanismos exatos para esses achados, nossos dados estão de acordo com a hipótese de uma lesão tecidual mediada por neutrófilos, associada aos maiores níveis de IL-17 durante o curso da DP. Essa citocina, presente em maiores quantidades, pode atrair neutrófilos para os sítios inflamados (OUYANG *et al*., 2008), além de ter a capacidade de aumentar a atividade das enzimas neutrofílicas,

como a protease neutrofílica e a MPO (YU *et al*., 2007). Além dessa função sobre neutrófilos, IL-17A pode também induzir a diferenciação de osteoclastos pela sua influência sobre fatores como RANKL e IL-1 (SATO *et al*., 2006). Tomados em conjunto, esses dados apontam que o polimorfismo de *IL-17A*, especialmente o alelo A, parece estar associado com uma maior expressão de IL-17A, recrutamento de neutrófilos, agravamento do padrão inflamatório, levando às piores condições clínicas observadas.

Em relação à citocina IL-10, existem na literatura muitos estudos conflitantes envolvendo polimorfismos dos genes desta citocina e a DP (BERGLUNDH *et al.*, 2002; MOREIRA *et al*., 2009; COSTA *et al.*, 2010; ZHONG *et al*., 2012). No presente estudo, encontramos que os pacientes com periodontite crônica apresentaram maior frequência do genótipo AA do polimorfismo *1087* da IL-10, em desacordo com estudos de Berglundh *et al.*, (2002) e Atanasovska-Stojanovska *et al.*, (2012), que mostraram uma associação da DP com o genótipo GG. Esses resultados conflitantes podem ser atribuídos a vários fatores, como as diferenças na definição da DP, a heterogeneidade da população, além de outros fatores de risco que podem estar sobrepostos. É interessante notar que, na comparação da produção de IL-10 entre os genótipos, os pacientes com DP e genótipo GG tiveram maior produção de IL-10. De fato, na literatura encontramos que o alelo G está associado a uma maior expressão de IL-10, enquanto o alelo A demonstra menor expressão de IL-10 (TURNER *et al.*, 2004).

Em relação ao polimorfismo de *TNFA* não encontramos nenhuma associação com a periodontite crônica em nosso estudo, corroborando os achados de vários outros autores (CRAANDIJK *et al.*, 2002; FASSMANN *et al.*, 2003; FOLWACZNY *et al.*, 2004; SAKELLARI *et al.*, 2006; MENEZES & COLOMBO, 2007; MOREIRA *et al*., 2009; COSTA *et al.*, 2010).

Do mesmo modo, o polimorfismo de *IL-17F* não foi relacionado com a doença periodontal em nosso estudo, apesar de algumas evidências que apontam a associação entre o polimorfismo da IL-17F em outras doenças inflamatórias, como a colite ulcerativa (ARISAWA *et al.*, 2008). Um estudo recente também não demonstrou diferenças entre pacientes com e sem doença periodontal referente à expressão dos genes *IL-17F* (SARAIVA *et al.*, 2012).

Em vista desses dados, é possível afirmar que o papel dos mediadores inflamatórios presentes na periodontite precisa ser interpretado dentro da complexidade da doença. O entendimento dos mecanismos envolvidos na patogênese da DP deve considerar uma série de fatores que se interligam. Dessa forma o estudo pontual de algumas citocinas pode não representar o quadro completo do dinamismo que ocorre nos tecidos periodontais frente à

agressão bacteriana. Entretanto, o estudo dos possíveis mecanismos genéticos que podem influenciar no equilíbrio saúde *versus* doença periodontal é importante para se desenvolver um maior domínio do complexo desenvolvimento das desordens periodontais e esclarecer os motivos de nem todos os indivíduos serem afetados igualmente. Sendo assim, apesar de alguns dados conflitantes, as citocinas estudadas demonstram papéis importantes dentro da patogênese da DP e a identificação de polimorfismos genéticos que podem afetar os níveis dessas citocinas nos tecidos periodontais é de suma importância. Este tipo de estudo permitirá a identificação de grupos de risco ou susceptibilidade baseados nas características genéticas. Já existe, por exemplo, um kit disponível para teste de susceptibilidade genética para doença periodontal, baseado na identificação de polimorfismos específicos da citocina IL-1a e IL-1b. Assim, após a definição de grupos de susceptibilidade, podem ser programadas medidas de prevenção ou intervenções terapêuticas precoces a fim de se evitarem as consequências desfavoráveis da doença.

## 5 CONCLUSÕES

Com base nos dados obtidos, é possível concluir que:

- Há associação entre os polimorfismos dos genes *IL-17A* e *IL-10* e a periodontite crônica;
- Não há associação entre os polimorfismos dos genes *IL-17F* e *TNFA* e a periodontite crônica;
- A presença do alelo A polimórfico de *IL-17A* está associado a piores escores clínicos e inflamatórios periodontais, maiores níveis de IL-17A e CXCL8/IL-8;
- O genótipo AG de *IL-10* está relacionado a um pior escore clínico e inflamatório periodontal. Em contrapartida, o genótipo GG apresenta relação com níveis aumentados de IL-10 e menor expressão de CXCL10.

## REFERÊNCIAS


ABRAHAM, L. J.; KROEGER K.M. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNFgene: relevance to disease. *J Leukoc Biol*. v. 66, n. 4, p. 562-6. 1999.

ALBANDAR, J. M. Epidemiology and risk factors of periodontal diseases. *Dent Clin North Am.* v. 49, n. 3, p. 517-532, 2005.

ALMAN, A. C. et al. Loss of alveolar bone due to periodontal disease exhibits a threshold on the association with coronary heart disease. *J Periodontol*., v. 82, n. 9, p. 1304-13, 2011.

ARISAWA, T. et al. The influence of polymorphisms of interleukin-17A and interleukin-17F genes on the susceptibility to ulcerative colitis. *Journal of Clinical Immunology*. vol. 28, no. 1, p. 44–49, 2008.

ARMITAGE, G.C. Development of a classification system for periodontal disease and conditions. *Ann Periodontol*, Chicago, v. 4, n. 1 p. 1-6, 1999.

BABEL, N. et al. Analysis of tumor necrosis factor-alpha, transforming growth factor beta, interleukin- 10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J Periodontol.* v. 77, n. 12, p. 1978–1983, 2006.

BAKER, P. J. The role of immune responses in bone loss during periodontal disease. *Microbes Infect*. v. 2, n. 10, p. 1181-92, 2000.

BERGLUNDH, T. et al. Association of the -1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. *J Clin Periodontol.* v. 30, n. 3, p. 249–254, 2003.

BOUGHMAN, J. A. et al. Phenotypic assessment of early onset periodontites in sibships. *J Clin Periodontol*. v. 19, n. 2, p. 223-239. 1992.

BRASIL. MINISTÉRIO DA SAÚDE. SECRETARIA DE ATENÇÃO À SAÚDE. DEPARTAMENTO DE ATENÇÃO BÁSICA. Projeto SBBrasil 2010: pesquisa nacional de saúde bucal - resultados principais. Brasília: Ministério da Saúde, 92 p., 2011.

BRETT, P. M. et al. Functional gene polymorphisms in aggressive and chronic periodontitis. *J Dent Res.* v. 84, n. 12, p. 1149–1153, 2005.

COSTA, A. M. et al. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. *Gerodontology.* v. 27, n. 1, p. 70-75, 2010.

COSTA, F. O. et al. Progression of periodontitis and tooth loss associated with glycemic control individuals under periodontal maintenance therapy: a 5-year follow-up study. *Journal of Periodontology*. v. e0, p. 1-15, 2012.

CULLINAN, M. P. Acquisition and loss of Porphyromons gingivalis, Actinobacillus actinomycetemcomitans and Prevotella intermedia over a 5-year period: the effect of a triclosan/copolymer dentifrice. *J Clin Periodontol*. v. 30, n. 6, p. 532–541, 2003.
CULLINAN M. P. et al. Progression of periodontal disease and interleukin-10 gene polymorphism. *J Periodontal Res*. v. 43, n. 3 p. 328-33, 2008.

COLIC, M. et al. Production of IL-10 and IL-12 by antigen-presenting cells in periapical lesions. *J Oral Pathol Med*, v. 39, n. 9, p. 690-696, 2010.

CRAANDIJK J. et al. Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *J Clin Periodontol*. v. 29, n. 1, p. 28-34, 2002.

DUTZAN, N. et al. Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-*κ* B ligand, interleukin (IL) -17, IL-10 and transforming growth factor-*β* during the progression of chronic periodontitis. *Journal of Clinical Periodontology*. v. 36, n. 5, p. 396–403, 2009.

ESPINOZA, J. L. et al. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. *PLoS One*. v. 6, n. 10, e26229, 2011.

FASSMANN, A. et al. Polymorphisms in the +252(A/G) lymphotoxin-alpha and the -308(A/G) tumor necrosis factor-alpha genes and susceptibility to chronic periodontitis in a Czech population. *J Periodontal Res*. v. 38, n. 4, p. 394-9, 2003.

FENG, Z.; WEINBERG, A. Role of bacteria in health and disease of periodontal tissues. *Periodontol 2000*. v. 40, n. 1, p. 50-76, 2006.

FIGUEREDO, C.M.; RIBEIRO, M.M. Aspectos imunológicos e genéticos no diagnóstico periodontal. In.: OPPERMANN, R.V.; RÖSING, C.K. *Periodontia – ciência e clínica*. 445 p São Paulo: Artes Médicas, 2001

FOLWACZNY, M. et al. Lack of association between the TNF alpha G -308 A promoter polymorphism and periodontal disease. *J Clin Periodontol.* v. 31, n.11, p. 449-53, 2004.

FOSSIEZ, F. et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med*. v. 183, n. 6, p. 2593-603, 1996.

GALBRAITH G. M. P. et al. Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *J Clin Periodontol*. v. 26, n. 11, p. 705–709, 1999.

GARLET, G. P. et al. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *Journal of Clinical Periodontology*, v. 31, n. 8, p. 671-679, 2004.

GARLET, G. P. *et al.* Cytokine pattern determines the progression of experimental periodontal disease induced by *Actinobacillus actinomycetemcomitans* through the modulation of MMPs, RANKL and their physiological inhibitors. *Oral Microbiol. Immunol.*, v. 21, n. 1, p. 12-20, 2006.

GARLET, G. P. et al. The dual role of p55 tumour necrosis factor-alpha receptor in Actinobacillus actinomycetemcomitans-induced experimental periodontitis: host protection and tissue destruction. *Clin Exp Immunol*, v. 147, n.1, p. 128-138, 2007.

GARLET, G. P. Destructive and protective roles of cytokines in periodontitis: a reappraisal from host defense and tissue destruction viewpoints. *J. Dent. Res.* v. 89, n. 12, p. 1349-1363, 2010.

GEMMELL, E.; YAMAZAKI, K.; SEYMOUR, G. J. Destructive periodontitis lesions are determined by the nature of lymphocytic response. *Crit Rev Oral*. v.13, n.1, p. 17-34, 2002.

GOMEZ, R.S.; DUTRA, W.O.; MOREIRA, P.R. Epigenetics and periodontal disease: future perspectives. *Inflamm Res*. v.58, n.10, p.625-629, 2009.

GONZALES, J. R. et al. Analysis of genetic polymorphisms at the interleukin-10 loci in aggressive and chronic periodontitis. *J Clin Periodontol*. v. 29, n. 12, 816–822, 2002.

GUROL, C. A comparative study of the role of cytokine polymorphisms interleukin-10 and tumor necrosis factor alpha in susceptibility to implant failure and chronic periodontitis. *Int J Oral Maxillofac Implants*. v. 26, n. 5, p. 955-60, 2011.

GRAVES, D.T.; COCHRAN, D.. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology.* v. 74, n. 3, p. 391-401, 2003.

GRAVES, D. T. Cytokines that promote periodontal tissue destruction. *Journal of Periodontology*. v. 79, n. Suppl 8, p. 1585-1591, 2008.

HONDA, T. et al. Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. *Clin Chim Acta*. v. 395, n. 1-2, p. 137-41, 2008.

JOHNSON, R. B.; WOOD, N.; SERIO, F. G. Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. *J Periodontol*. v. 75, n. 1, p. 37-43, 2004.

KAWAGUCHI, M. et al. IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. *J Allergy Clin Immunol*. v. 117, n. 4, p. 795-801, 2006.

KINANE, D. F. et al. Analysis of genetic polymorphisms at the interleukin-10 and tumour necrosis factor loci in early-onset periodontitis. *J Periodont Res.* v. 34, n. 7, p. 379–386. 1999.

KINANE, D.F.; BERGLUNDH, T.; LINDHE, J. Interações entre parasita e hospedeiro na doença periodontal. In: LINDHE, Tratado de Periodontia Clínica e Implatodontia Oral. 3 ed., Guanabara-Koogan, cap. 5, p. 148-175, 2005.

KINANE, D. F.; PRESHAW, P. M.; LOOS, B.G. Working Group 2 of Seventh European Workshop on Periodontology. Host-response: understanding the cellular and molecular mechanisms of host-microbial interactions--consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol*. v. 38 n. Suppl 11, p. 44-8, 2011.

KIM, E. S. et al. Interactions between IL17A, IL23R, and STAT4 polymorphisms confer susceptibility to intestinal Behcet's disease in Korean population. *Life Sciences*. v. 90, n. 19-20, p. 740–746, 2012.

KOTAKE, S. et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J Clin Invest*. v. 103, n. 9, p. 1345-52, 1999.

LAINE, M. L.; LOOS, B.G.; CRIELAARD, W. Gene polymorphisms in chronic periodontitis. *Int J Dent*. 2010 Epub 2010 Feb 9.

LIN, L.; PAN, Y. P.; YIN, L. Y. Study on the correlation of cytokine gene ppolymorphism with chronic periodontitis. *Shanghai Kou Qiang Yi Xue*. v. 12, n. 6, p. 456-9, 2003.

LOOS, B. G.; JOHN, R. P.; LAINE, M. L. Identification of genetic risk factors for periodontitis and possible mechanisms of action. *Journal of Clinical Periodontology*. v. 32, n. Suppl. 6, p. 159–179, 2005.

MADIANOS, P. N.; BOBETSIS, G. A.; KINANE, D. F. Is periodontitis associated with an increased risk of coronary heart disease and preterm and/or low birth weight births? *J Clin Periodontol*. v. 29, n. Suppl 3, p. 22-36, 2002.

MENEZES, N. G.; COLOMBO, A. P. Lack of association between the TNF-alpha -308 (G/A) genetic polymorphism and periodontal disease in Brazilians. *Braz Oral Res*. v. 22, n. 4, p. 322-7, 2008.

MENG, H. et al. Determinants of host susceptibility in aggressive periodontitis. *Periodontol 2000*. v. 43, n. 1, p. 133-159, 2007.

MERCADO, F. B. et al. Relationship between rheumatoid arthritis and periodontitis. *J. Periodontol*. v. 72, n. 6, p. 779-787, 2001.

MICHALOWICZ, B. S et al. Periodontal findings in adult twins. *Journal of Periodontology*. v. 62, n. 5, p. 293-99, 1991.

MICHALOWICZ, B. S. et al. Periodontal bacteria in adult twins. *Journal of Periodontology***.** v. 70, n. 3, p. 263-273, 1999.

MILLER, M. C.; MOHRENWEISER, H. W.; BELL, D. A. Genetic variability in susceptibility and response to toxicants. *Toxicology Letters*. v. 120, n. 1-3, p. 269-80, 2001.

MOREIRA, P. R. et al. TNFA and IL10 gene polymorphisms are not associated with periodontitis in Brazilians. *Open Dent J*. v. 7, n. 3, p. 184-90, 2009.

NARES, S. The genetic relationship to periodontal disease. *Periodontology 2000*. v. 32, n. 1, 36–49, 2003.

NORDANG, G. B. N. et al. Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand. *Rheumatology*. v. 48, n. 4, p. 367–370, 2009.

OFFENBACHER, S. Periodontal disease: pathogenesis. *Ann Periodontol*, v. 1, n. 1, p. 821-878, 1996.

OHYAMA, H. N. et al., The involvement of IL-23 and the Th 17 pathway in periodontitis. *Journal of Dental Research*. v. 88, n. 7, p. 633–638, 2009.

OUYANG, W. et al. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity*. v. 28, n. 4, p. 454–467, 2008.

OZÇAKA, O.; NALBANTSOY, A.; BUDUNELI, N. Interleukin-17 and interleukin-18 levels in saliva and plasma of patients with chronic periodontitis. *J Periodontal Res*. v. 46, n. 5, 592-8, 2011.

PAGE, R.C. Advances in the pathogenesis of periodontitis; summary of developments, clinical implications and future directions. *Periodontol 2000*. v.14, n.1, p. 216-248, 1997.

PARKHILL, J. M. et al. Association of interleukin-1 gene polymorphisms with early onset periodontitis. *J Clin Periodontol*. v. 27, n. 9, p. 682-689, 2000.

PEREA, E. J. Oral flora in the age of molecular biology. *Med Oral Patol Oral Cir Bucal*. v. 9, n. 6-10, p. 1-5, 2004.

QUEIROZ-JUNIOR, C. M. et al. Experimental arthritis triggers periodontal disease in mice: involvement of TNF-α and the oral Microbiota. *J Immunol*. v. 187, n. 7, p. 3821-30, 2011.

QUEIROZ-JUNIOR, C. M. et al. Experimental arthritis exacerbates Aggregatibacter actinomycetemcomitans-induced periodontitis in mice. *J Clin Periodontol*. v. 39, n. 7, p. 608-16, 2012.

RICCI, M. et al., Association between genetic risk score and periodontitis onset and progression: A pilot study. *archives of oral biology*. v. 56, n. 12, p. 1499 – 1505. 2011.

ROUSSET, F. et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA*. v. 89, n. 5, p. 1890–3, 1992.

SANZ, M.; WINKELHOFF, A. J. Working Group 1 of Seventh European Workshop on Periodontology. Periodontal infections: understanding the complexity—consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol*. v. 38, n. suppl 11, p. 3-6, 2011.

SARAIVA, A.M. et al. Evaluation of IL17A expression and of IL17A, IL17F and IL23R gene polymorphisms in Brazilian individuals with periodontitis. *Hum Immunol,* 2012.

SASAKI, H. et al. The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalisinduced alveolar bone loss. *Journal of Periodontal Research,* v. 39, n. 6, p. 432-441, 2004.

SATO, K. et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *Journal of Experimental Medicine*. v. 203, n. 12, p. 2673–2682, 2006.

SAKELLARI, D. et al. No correlation of five gene polymorphisms with periodontal conditions in a Greek population. *J Clin Periodontol.* v. 33, n. 11, p. 765-70, 2006.

SCAREL-CAMINAGA, R. M. et al. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. *J Clin Periodontol.* v. 31, n. 6, p. 443–448, 2004.

SCHENKEIN, H. A. et al. IL-17 in sera from patients with aggressive periodontitis. *J Dent Res.* v. 89, n. 9, p. 943-7, 2010.

SUMER, A. P. et al. Association of interleukin-10 gene polymorphisms with severe generalized chronic periodontitis. *J Periodontol.* v. 78, n. 3, p. 493-7, 2007.

TAYLOR, J. J. et al. Cytokine gene polymorphism and immunoregulation in periodontal disease. *Periodontology 2000*. v. 35, n. 1, p. 158-182, 2004.

TERVONEN, T, et al. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. *J Clin Periodontol*. v. 34, n. 5, p. 377-83, 2007.

VERNAL, R. et al. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. *Journal of Clinical Periodontology*. v. 32, n. 4, p. 383–389, 2005.

WANG, L. et al. Association analysis of IL- 17A and IL-17F polymorphisms in Chinese han women with breast cancer. *PLoS ONE*, v. 7, n. 3, e34400, 2012.

WILLIAMS, R. C.; PAQUETTE, D. W. Understanding the pathogenesis of periodontitis: a century of discovery. *J Int Acad Periodontol.* v. 2, n. 3, p. 59-63, 2000.

WILSON, A. G. et al. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA.* v. 94, n. 7, p. 3195-99. 1997.

WU, X. et al. Association between polymorphisms in interleukin-17A and interleukin-17F genes and risks of gastric cancer. *International Journal of Cancer*, v. 127, n. 1, p. 86–92, 2010.

YAMAZAKI, K. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *J Clin Periodontol*. v. 28, n. 9, p. 828-32, 2001.

YU, J. J. et al. An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals. *Blood*. v. 109, n. 9, p. 3794–3802, 2007.

ZHANG, X.; TENG, Y.T. Interleukin-10 inhibits gram-negative-microbe-specific human receptor activator of NF-kappaB ligand-positive CD4+-Th1-cell-associated alveolar bone loss in vivo. *Infectology Immunity.* v. 74, n. 8, p. 4927-4931, 2006.

ZHONG, Q. et al. Interleukin-10 gene polymorphisms and chronic/aggressive periodontitis susceptibility: a meta-analysis based on 14 case-control studies. *Cytokine*. v. 60, n. 1, p. 47-54, 2012.

ANEXO A – Parecer do Comitê de Ética

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 324/08

Interessado(a): Profa. Tarcília Aparecida da Silva
DCPCO
Faculdade de Odontologia - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 27 de agosto de 2008, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Expressão de mediadores inflamatórios na doença periodontal humana"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antônio Carlos, 6627 - Unidade Administrativa II - 2º andar - Sala 2005 - (31) 3409-4592 - 31270-901 - BELO HORIZONTE
Telefax: (031) 3409-4592 - coep@prpq.ufmg.br