Universidade Norte do Paraná
UNOPAR

CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
MESTRADO EM ODONTOLOGIA

MIULA PORTELINHA BRAGA

ANÁLISE DA ASSOCIAÇÃO DOS POLIMORFISMOS NOS GENES *IL6*-174 E *TNFα*-308 COM A PERIODONTITE CRÔNICA EM IDOSOS COM E SEM DIABETES MELLITUS (TIPO 2).

Londrina
2012

MIULA PORTELINHA BRAGA

ANÁLISE DA ASSOCIAÇÃO DOS POLIMORFISMOS NOS GENES *IL6*-174 E *TNFα*-308 COM A PERIODONTITE CRÔNICA EM IDOSOS COM E SEM DIABETES MELLITUS (TIPO 2).

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Norte do Paraná - UNOPAR, como requisito parcial à obtenção do título de Mestre em Odontologia.

Área de Concentração: Dentística Preventiva e Restauradora

Orientadora: Profª Drª Regina Célia Poli-Frederico
Co-orientadora: Profª Drª Sandra Kiss Moura

Londrina
2012

Dados Internacionais de catalogação-na-publicação
Universidade Norte do Paraná
Biblioteca Central
Setor de Tratamento da Informação

Braga, Miula Portelinha
R794r Análise da associação dos polimorfismos nos genes *IL6*-174 e *TNFα*-308 com a periodontite crônica em idosos com e sem diabetes mellitus (Tipo2)/ Miula Portelinha Braga. Londrina : [s.n], 2012.
x; 46p.

Dissertação (Mestrado). Odontologia. Dentística Preventiva e Restauradora. Universidade Norte do Paraná.
Orientador: Profª Drª. Regina Célia Poli-Fredrico

1- Odontologia - dissertação de mestrado – UNOPAR 2- Doença periodontal 3- Polimorfismo genético 4- Interleucina 5- Fator de necrose tumoral alfa 6- Idosos I- Poli-Fredrico, Regina Célia, orient. II- Universidade Norte do Paraná.

CDU 616.314-089.27/.28

MIULA PORTELINHA BRAGA

ANÁLISE DA ASSOCIAÇÃO DOS POLIMORFISMOS NOS GENES *IL6*-174 E *TNFα*-308 COM A PERIODONTITE CRÔNICA EM IDOSOS COM E SEM DIABETES MELLITUS (TIPO 2).

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Norte do Paraná - UNOPAR, como requisito parcial à obtenção do título de Mestre em Odontologia.

Área de Concentração: Dentística Preventiva e Restauradora

BANCA EXAMINADORA

Profa Dra Orientadora
Regina Celia Poli- Frederico
UNOPAR

Profa Dra Componente da Banca
Sandra Mara Maciel
UNOPAR

Profa Dra Componente da Banca
Paula Cristina Trevilatto
PUC-PR

Londrina, 24 de fevereiro de 2012.

DEDICATÓRIA

Ao meu Deus:

Pelo dom de vida e pelo conforto
nas horas mais difíceis.

Aos meus pais Airton e Mônica :

Por me amarem além dos limites desta vida,
por todos os ensinamentos valiosos e
pela luta incansável para minha
felicidade e sucesso.

Aos meus amados irmãos Arthur e Milian:

Por serem o meu orgulho,
minha preocupação e
minha diversão.

AGRADECIMENTOS

À Professora Orientadora Regina Célia Poli-Frederico:

Pelo incentivo, carinho e amizade conferidos
a mim nesses anos de convivência e
por ser profissional que inspira, que me faz buscar
a excelência.

À Profª Drª Sandra Mara Maciel :

Por todos os ensinamentos transmitidos como mestra,
pelo companheirismo, gentileza
e sabedoria.

À Profª Drª Sandra Kiss Moura :

Pelos divertidos conselhos,
por encorajar e acreditar na superação de seus alunos.

À querida Sibele Cardoso:

Obrigada pela companhia, ajuda e incentivo
durante os dias de estudos no laboratório de Biologia Molecular

Aos idosos integrantes da pesquisa:

Pela confiança e fundamental participação.

À banca examinadora:

Pela troca de ideias e experiências durante a qualificação
e defesa que enriqueceram esta dissertação.

Muito Obrigada!

BRAGA, Miula Portelinha. **Análise da associação dos polimorfismos da IL6-174 e TNFA-308 com a periodontite crônica em idosos com e sem diabetes mellitus (Tipo 2).** 47 fls. [Dissertação de Mestrado]. Programa de Pós-Graduação em Odontologia – Universidade Norte do Paraná, Londrina,- Universidade Norte do Paraná, Londrina, 2012.

RESUMO

A doença periodontal (DP) é uma doença crônica multifatorial relacionada a um processo inflamatório que pode levar à destruição dos tecidos de suporte dos dentes. Diabetes mellitus Tipo 2 (T2DM) é uma doença complexa resultante da resistência à insulina combinada com uma incapacidade de produzir mais insulina na quantidade suficiente para compensar a resistência à insulina. Fatores genéticos e ambientais estão envolvidos na etiologia destas doenças. Este estudo propõe avaliar a associação entre a doença periodontal e os polimorfismos genéticos dos genes IL6-174 e TNFA-308 em idosos com e sem diabetes mellitus Tipo 2 do município de Londrina, PR. Uma amostra de 191 idosos fisicamente independentes foram avaliados quanto presença de doença periodontal através do índice PIP (Índice de Perda de Inserção Periodontal), a glicose e a hemoglobina glicosilada (HbA1c) foram analisados por métodos laboratoriais de rotina. Os idosos foram classificados em quatro grupos: grupo controle (n = 56), grupo periodontite crônica (CP) (n = 79), grupo diabetes mellitus tipo 2 (T2DM) (n = 19) e CP & T2DM (n = 37). O polimorfismo genético foi analisado por meio da reação em cadeia da polimerase seguida da clivagem com enzima de restrição. Os produtos de digestão foram visualizados através da eletroforese em gel de agarose (2%). A análise estatística foi realizada usando o modelo de regressão logística para testar a associação entre a DP em idosos e polimorfismo nos genes *IL6* e *TNFA*. Covariantes sociodemográficas e hábito tabagista também foram incluídos no modelo. O teste Qui-quadrado foi empregado para a análise da relação entre os genótipos dos polimorfismos da *IL6* e *TNFA*. O nível de significância foi de $p < 0,05$. Houve significativa associação para o polimorfismo no gene da *IL6*, gênero e hábito de fumar entre os idosos estudados com a DP. Sugere-se que a frequência do alelo C do gene da IL6, seja um fator protetor a periodontite crônica (P = 0,02, OR = 0,468, IC 95%: 0,243-0,904) e como fator de risco à diabetes (P = 0,02, OR = 2,254, IC 95%: 1,161-4,375). Não houve associação estatisticamente significante entre o polimorfismo no gene *TNFA* e os idosos estudados, sugerindo que mais pesquisas são necessárias para melhorar a compreensão dos mecanismos que regulam a periodontite e os polimorfismos genéticos no referido gene.

Palavras-chave: Doença Periodontal. Suscetibilidade genética. Interleucina 6. Fator de necrose tumoral alfa. Idosos.

BRAGA, Miula Portelinha. **Association among interleukin 6 gene polymorphism, chronic periodontitis and diabetes in elderly Brazilians.** 47 fls. [Dissertação de Mestrado]. Programa de Pós-Graduação em Odontologia – Universidade Norte do Paraná, Londrina,- Universidade Norte do Paraná, Londrina, 2012.

ABSTRACT

Periodontal disease (PD) is a chronic multifactorial disease related to an inflammatory process that can lead to the destruction of the tissues supporting the teeth. Type 2 diabetes mellitus (T2DM) is a complex disease resulting from resistance to insulin combined with a failure to produce enough additional insulin to compensate for the insulin resistance. Genetic and environmental factors are involved in this disease. This study aims to evaluate the association between periodontal disease and genetic polymorphism in the elderly independent of Londrina, PR. 191 physically independent elderly were evaluated periodontal disease through the PIP Index (Index Periodontal Attachment Loss), the glucose and hemoglobin glycosylated (HbA1c) were analyzed by routine laboratory methods. The elderly were classified into four groups: control group (n = 56), chronic periodontitis (CP) group (n = 79), type 2 diabetes mellitus (T2DM) group (n = 19) and CP & T2DM group (n = 37). The genetic polymorphism was analyzed by polymerase chain reaction followed by cleavage with restriction enzyme. The digestion products were visualized by electrophoresis in agarose gel (2%). Statistical analysis was performed using logistic regression model to test the association between PD in the elderly and polymorphism in the genes *IL6* and *TNF-a*. Sociodemographic covariates and smoking habits were also included in the model. The chi-square test was used to analyze the relationship between the genotypes of the polymorphisms of *IL6* and *TNF-a.* The significance level was $p < 0.05$. There was a significant association for the polymorphism of the *IL6* gene, gender and smoking habits among the aged. It is suggested that the frequency of C allele of the *IL6* gene, is a protective factor to chronic periodontitis (P = 0.02, OR = 0.468, 95% CI: 0.243 to 0.904) and as a risk factor for diabetes (P = 0.02, OR = 2.254, 95% CI: 1.161 to 4.375). There was no statistically significant association between the polymorphism in *TNF-a* in the elderly studied, suggesting that more research is needed to improve understanding of the mechanisms that regulate periodontitis and genetic polymorphisms in that gene.

Keywords: Periodontal Disease. Genetic susceptibility. Interleukin 6. Tumor necrosis factor alpha. Elderly.

SUMÁRIO

1 INTRODUÇÃO

A população brasileira vem envelhecendo de forma acelerada a partir da década de 60[1]. De 3 milhões em 1960, as pessoas com 60 anos ou mais passaram a ser 17 milhões em 2006, caracterizando um aumento de 600%[1]. Em 2020 o Brasil será o sexto país do mundo em número de idosos, com um contingente superior a 30 milhões de pessoas[2].

Para os idosos, proteção e promoção da função mastigatória é essencial para manter a qualidade de vida física e social. Como é conhecido, no entanto, as populações de idosos em muitos países muitas vezes mostram altos índices de edentulismo e muitas vezes apresentam apenas alguns dentes funcionais[3].

A destruição periodontal é uma frequente experiência entre os idosos[4,5]. É um fator primário que contribui para a perda de aproximadamente um em cada cinco dentes entre adultos em populações ocidentais[6-12]. Também contribui para cerca de 40% das extrações[13]. É relevante coletar e analisar dados sobre a progressão da doença periodontal em pessoas idosas, afim de identificar aqueles que são propensos a perder os dentes[14].

A doença periodontal (DP) é uma doença crônica multifatorial relacionada a um processo inflamatório que leva à destruição dos tecidos de suporte dos dentes[15]. Ela é iniciada e mantida por fatores oriundos do biofilme bacteriano subgengival[16]. Com o acúmulo de biofilme nas superfícies dos dentes, as células do periodonto entram em contato com produtos bacterianos e produzem citocinas pró-inflamatórias e outros mediadores químicos da inflamação[17], que iniciam a resposta inflamatória no interior dos tecidos periodontais.

As citocinas, tais como*:* IL-6 e o fator de necrose tumoral (TNF) são proteínas, secretadas por muitos tipos celulares, que agem como mensageiras, iniciando e mantendo as respostas imunes e inflamatórias, regulando o crescimento e diferenciação das células[18].

Embora a placa bacteriana seja essencial para o desenvolvimento das doenças periodontais, sua presença por si só, não resulta necessariamente na destruição do periodonto[19].

A influência genética sobre a DP crônica tem sido amplamente estudada nos últimos anos[20-22] e a busca sobre o papel dos genes e de suas variantes (polimorfismos) pode ajudar a elucidar a patogênese e a progressão da doença[22]. Os polimorfismos genéticos podem resultar em alterações nas proteínas (citocinas) ou na sua expressão, culminando possivelmente em alterações no sistema imune podendo ser determinantes na evolução da DP. Doenças com etiopatologia complexa, como a DP, são poligênicas[23] e polimorfismos nesses genes podem proteger o hospedeiro ou contribuir para o desenvolvimento da doença.

Estudos recentes listaram genes polimórficos relacionados à doença periodontal[24,25], entre eles estão: o gene codificador da Interleucina 6 *(IL6)* e do fator de necrose tumoral α (TNF-α).

A investigação sobre estes genes e seus polimorfismos em idosos pode ampliar os conhecimentos sobre a influência genética e a susceptibilidade à doença peridontal.

2 REVISÃO DE LITERATURA

2.1 A DOENÇA PERIODONTAL

A doença periodontal (DP) é a segunda doença bucal mais prevalente no mundo[26]. Dados epidemiológicos disponíveis na Organização Mundial de Saúde (OMS) mostram que a prevalência da doença periodontal e gravidade tendem a ser elevadas nos grupos etários mais velhos, em comparação com grupos etários mais jovens[27]. No Brasil a prevalência da doença periodontal mostrou-se alta em todas as faixas etárias, com menos de 8% dos idosos, entre 65 a 74 anos, apresentando gengivas sadias e as proporções mais favoráveis para essa faixa etária foram encontradas na Região Sul[28]. Estas informações foram confirmadas recentemente através do último levantamento epidemiológico realizado no Brasil sobre as condições de saúde bucal da população, indicando que o percentual de indivíduos sem nenhum problema periodontal foi de 68% para a idade de 12 anos, 51% para a faixa de 15 a 19 anos, 17% para os adultos de 35 a 44 anos e somente 1,8% nos idosos de 65 a 74 anos. Cabe ressaltar que nos idosos, os problemas periodontais

tiveram pequena expressão em termos populacionais, em decorrência do reduzido número de dentes presentes[29].

A doença periodontal (DP) consiste da quebra de homeostasia do tecido periodontal, através da agressão primária do biofilme dentário[30].

O biofilme microbiano dentário é reconhecido como uma comunidade de bactérias, instalada em uma matriz constituída por polímeros extracelulares, aderidos entre si ou a uma superfície sólida, como esmalte, dentina, cemento, próteses, implantes, entre outras[31].

As bactérias presentes nessa estrutura agridem os tecidos, que podem responder com uma reação imunoinflamatória de acordo com os reflexos da resposta do hospedeiro e a capacidade patogênica da microbiota, definindo o tipo e a severidade da DP. Portanto, para ocorrer a DP, as bactérias devem ser virulentas e superar as defesas do hospedeiro[32].

As células gengivais em contato com os produtos bacterianos produzem mediadores inflamatórios denominados citocinas[33]. Interleucinas (IL) e fatores de necrose tumoral (TNF) são alguns dos mediadores inflamatórios encontrados em níveis elevados no fluido crevicular gengival e tecidos gengivais quando há DP, que os tornam importantes nos testes diagnósticos e de susceptibilidade[34].

Fatores ambientais, nível socioeconômico ou comportamental são também classificados como fatores de risco e podem apresentar significante relação com as doenças periodontais [35]. Diversos estudos relatam que o tabagismo também pode influenciar a saúde periodontal, dessa forma, suspeita-se que os fumantes tenham maior risco em desenvolver DP[36-38].

Depressão, estresse e alguns fatores psicossociais também têm sido relacionados a uma possível relação na quebra da homeostasia das células do periodonto saudável e, por consequência, ao desenvolvimento da DP[39]. Alguns estudos relacionam o Diabetes mellitus Tipo 2 ao aumento e severidade à DP[40-42].

Há diferentes doenças que afetam o sistema de suporte dos dentes, mas a forma mais prevalente é a periodontite crônica[18] que de acordo com o grau de perda de inserção, pode ser classificada em periodontite leve, moderada ou severa, caracterizada respectivamente por perda de inserção de 2 a 4 mm; 4 a 7 mm e acima de 7 mm[41].

A identificação e o manejo correto desses fatores de risco, bem como o conhecimento dos processos clínicos e imunopatológicos da DP, podem ajudar no desenvolvimento de estudos mais aprofundados sobre o tema.

2.2 POLIMORFISMOS GENÉTICOS NAS DOENÇAS PERIODONTAIS

Estudos realizados em gêmeos demonstram que, tanto os fatores ambientais como os genéticos estão relacionados ao desenvolvimento, gravidade e suscetibilidade à doença periodontal[42,43]. Fatores de risco para muitas doenças, incluindo doenças periodontais, não são iguais para todos os indivíduos[44,45]. Estes podem responder diferentemente aos desafios ambientais comuns e cada resposta pode ser influenciada pelo perfil genético individual. Especificamente, as diferentes formas de genes (variantes alélicas) podem produzir variações na estrutura do tecido (imunidade inata), as respostas de anticorpos (imunidade adaptativa) e em mediadores inflamatórios (não específicas da inflamação). Variantes alélicas de vários e diferentes locos gênicos provavelmente influenciam na suscetibilidade à DP[46.] As doenças mais comuns, assim como a DP, possuem uma etiologia genética complexa, pois não são oriundas de um único defeito em um único gene[46]. Além disso, os aspectos da resposta inflamatória, ou seja, os mediadores inflamatórios (citocinas) secretados pelos linfócitos T helper (Th) e polimorfismos nesses genes tem atraído atenção pela possibilidade de influenciar na resposta do hospedeiro à DP[47]. Estas substâncias são, portanto, consideradas marcadores biológicos para a doença periodontal[48].

2.2.1 Interleucina 6 (*IL6*)

A IL-6 é uma citocina produzida por muitos tipos celulares, como monócitos estimulados, os fibroblastos, células endoteliais e linfócitos T e B[49], com diversas funções: diferenciação e / ou ativação de macrófagos e células T; crescimento e diferenciação de células B; estimulação da hematopoiese; diferenciação neural[50], estimulador da diferenciação dos osteoclastos e inibidor da formação óssea[51].

Na periodontite, a IL-6 é expressa por uma variedade de células na lesão periodontal e, em comum com a IL-1, atua no aumento da reabsorção óssea[52,53]. Tem sido demonstrado que elevados níveis de IL-1β e IL-6

são induzidos por patógenos periodontais e são correlacionados com a contínua destruição de tecido observada na periodontite[54].

Trevilatto et al. (2003)[24] demonstraram que o genótipo GG do polimorfismo da IL-174 pode estar associado à periodontite crônica em uma população caucasiana do Brasil e D'Aiuto et al. (2004)[55], em Londres, observaram que, em pacientes que apresentavam graves infecções periodontais, o aumento da IL-6 foi associado com a presença do alelo C. A frequência dos polimorfismos pode variar entre grupos étnicos[56].

2.2.2 Fator de necrose tumoral alfa (TNFA)

O TNFA é uma citocina pró-inflamatória, que tem sido detectada no tecido, no fluido gengival[57,58] e no plasma sanguíneo[59,60] de indivíduos com periodontite. Esta citocina é um mediador imunológico que, além de seu efeito inflamatório, aumenta a reabsorção óssea e regula a proliferação dos fibroblastos[61]. A atividade do TNFA é regulada pela interleucina 10 (IL10) e outras moléculas anti-inflamatórias[25], sugerindo que alguma deficiência nesse mecanismo de regulação pode ser relacionada com a DP[45].

Galbraith et al. (1998)[62] compararam as frequências genotípicas do *TNFA* em pacientes com periodontite crônica entre pacientes sadios (grupo controle) e não encontraram diferenças entre os grupos. Recentemente, Craandijk et al. (2002)[63] também não encontraram associações significativas entre uma série de quatro diferentes polimorfismos no gene *TNFA* e pacientes com periodontite.

Moreira et al.(2009)[25] avaliaram o polimorfismo genético na posição -308 do *TNFA* em uma amostra de indivíduos brasileiros com diferentes formas e severidade de DP, mas também não obtiveram associação entre o polimorfismo do *TNFA* e a DP.

Relacionar o polimorfismo dos genes codificadores das Interleucinas e *TNF-α* com a doença periodontal, em idosos brasileiros, pode contribuir na compreensão do processo de desenvolvimento e incidência da DP nesse grupo populacional.

2.3 POLIMORFISMOS GENÉTICOS E O DIABETES MELLITUS

Diabetes mellitus tipo 2 (T2DM) é uma doença complexa resultante da resistência à insulina combinada com uma incapacidade de produzir mais insulina na quantidade suficiente para compensar a resistência à insulina[64].

T2DM pode surgir a partir de uma interação entre fatores ambientais, como obesidade, sedentarismo, a ingestão de alimentos ricos em calorias e susceptibilidade genética que pode resultar no aumento da manifestação clínica da doença[65].

Embora o T2DM seja uma doença complexa e os fatores ambientais podem iniciar e modificar o seu desenvolvimento, os polimorfismos genéticos de citocinas também podem estar envolvidos na manifestação da doença [66, 67].

Diabetes mellitus aumenta o risco para o desenvolvimento de doença periodontal[68, 69].

Vários caminhos estão sendo investigados para tentar explicar a relação entre diabetes e doença periodontal, incluindo a resposta celular, hiperglicemia e polimorfismos genéticos, mas os mecanismos de interação entre as duas doenças ainda não estão claros[70, 71].

3 PROPOSIÇÃO

O objetivo do trabalho foi investigar a associação dos polimorfismos nos genes *IL6* (G-174C) e *TNFα* (C-308A) com a doença periodontal crônica em idosos com e sem diabetes mellitus Tipo 2.

4 ARTIGO

"Association among interleukin 6 gene polymorphism, chronic periodontitis and diabetes in elderly Brazilians".

Este artigo será submetido à publicação no periódico European Journal of Oral Sciences- ISSN 1600-0722

Association among interleukin 6 gene polymorphism, chronic periodontitis and diabetes in Brazilians elderly

M. P. Braga[a], S. K. Moura[b], S. M. Maciel, A.S. Marquez, R.C. Poli-Frederico[c,*]

[a]DDS, Department of Restorative Dentistry, Faculty of Dentistry, University of North of Parana, Londrina, PR, Brazil

[b]DDS, MS, PhD, Department of Restorative Dentistry, Faculty of Dentistry, University of North of Parana, Londrina, PR, Brazil

[c]MS, PhD, Department of Genetics and Molecular Biology, Faculty of Dentistry, University of North of Parana, Londrina, PR, Brazil

***Corresponding author**

Regina Célia Poli-Frederico

Universidade Norte do Paraná, Faculdade de Odontologia

Rua Marselha 183, Jardim Piza, Londrina, PR, Brasil. CEP 86041-120

Phone: +55-43-3371-7820 Fax: +55-43-3371-7741

E-mail: reginafrederico@yahoo.com.

Abstract

This study aims to evaluate the possible associations between genetic polymorphism for interleucina 6 (*IL6)* and tumor necrosis factor-alpha (*TNFα*), periodontitis and diabetes in the Brazilians elderly. 191 subjects selected were evaluated for CP using the PIP index (Index of Periodontal Attachment Loss). Conditions such as gender, age and smoking status were evaluated through of a structured questionnair. The analysis of the polymorphism of *IL6* -174 and *TNFα*-308 was performed by PCR-RFLP from the DNA extraction from blood leukocytes of each elderly. Glucose and glycosylated hemoglobin (HbA1c) were analyzed by routine laboratory methods. All elderly were classified into four groups: healthy control group (n=56), chronic periodontitis (CP) group (n=79), Type 2 diabetes mellitus (T2DM) group (n=19) and CP&T2DM group (n=37). The genotypes for the two genes were in Hardy-Weinberg equilibrium (each P-value> 0.05). The C-allele of IL6 (-174) gene might be a protective factor against of CP (*P*= 0.02, OR= 0.468, 95% CI: 0.243-0.904). The variable female gender may a protective factor against of CP (P<0.01, OR= 0.293, 95% CI:0.142-0.584) and allele C be may risk factor to T2DM (P = 0.02, OR= 2.254, 95% CI: 1.161-4.375). No significant differences were found for the *TNFA* genotype distribution or the alleles frequencies between the genes with and not CP or T2DM. It was concluded that male gender, tabagism and polymorphism IL6/G-174C were associated with chronic periodontitis in the elderly studied also, allele C of polymorphism IL6-174 was associated with diabetes in this population.

Keywords: Chronic Periodontitis. Diabetes Mellitus. Interleukin-6. Tumor Necrosis Factor-alpha. Aged.

Introduction

For elderly people, protecting and promoting masticatory function is essential to maintain good physical and social quality of life [1]. Studies on such groups will contribute both to the planning of appropriate care and to monitoring of the overall effects of oral care services in a given population [2]. Periodontal destruction is a primary factor contributing to the loss of approximately one in five teeth among adults in Western populations [3- 8] and is a frequent experience among elderly people [9, 10].

Chronic periodontitis (CP) is considered as complex disease associated with inflammation and relating to multiple genes [11-13]. The reported risk factors for periodontal disease progression are age, periodontal pathogens, smoking and systemic diseases such as diabetes, which have also been identified as risk indicators in numerous cross-sectional studies, among older adults [14-17] as well as genetic factors [18].

Type 2 diabetes mellitus (T2DM) is a complex disease resulting from resistance to insulin combined with a failure to produce enough additional insulin to compensate for the insulin resistance [19] and increases the risk of periodontal disease [20, 21]. Several ways have been investigated to explain the relationship between diabetes and periodontal disease, including cellular and microbiological response and genetic markers [22-26] but the interaction mechanisms between diabetes and periodontal disease are still not clear [27, 28].

Polymorphisms in genes encoding molecules of the host defense system, such as cytokines, have been targeted as potencial genetic markers to CP [29]. Many studies have focused on the potencial role of the pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor-alfa (TNF-α) in chronic periodontitis [25, 30- 32].

Interleukin-6 (IL-6) plays a role in B-cell differentiation and T-cell proliferation[33], stimulates hematopoiesis [34], accelerates bone resorption [35] and inhibits the bone formation [36].

The locus *IL6 -174* G/C(rs1800795) genetic polymorphism was found in relation to chronic periodontitis [31, 37]. TNF-α has diverse functions, induces the secretion of collagenase by fibroblasts, stimulates resorption of cartilage and bone, and has been implicated in the destruction of periodontal tissue in periodontitis [38, 39,40]. Polymorphisms in the promoter region of the *TNFα* gene at position -308 G/A (rs1800629) have been evaluated [41, 42]. However, there are conflicting results on the functional relevance of this polymorphism to justify investigation in this area to assess the possible association between the -308 G/A polymorphism and periodontitis [43]. The genetic variation in cytokine production may explain some of the differences among individuals in the progression of complex diseases [19]. This study aims to evaluate the possible associations between the genetic polymorphisms for interleukin 6 (*IL6-174*), tumor necrosis factor alfa (*TNFα-308*), periodontitis and diabetes in the elderly independent of Londrina-Pr.

Materials and methods

Subjects Selection

A convenience sample of 191 subjects ≥ 60 years of age (mean age 67.8) were recruited for study from the Age and Longevity (EELO) cross-sectional study (approved by the Ethical Committee in Research at UNOPAR, protocol 0253/11). The subjects are from the South region of Brazil and all participants gave informed written consent.

The study included individuals of both genders who have independent living, which are classified in levels 3 and 4 proposed by the Spirduso (2005) [45]. Subjects did not have any of following exclusion criteria: diseases of the oral hard and soft tissues except caries and periodontal disease; use of orthodontic appliances; need for pre-medication for dental disease; chronic usage of anti-inflammatory drugs, known systemic diseases except for diabetes; hepatitis or HIV infection and any illness or limitation that would prevent the testing such as physical and mental disabilities.

Each participant was required to complete a questionnaire, containing gender, age, ethnicity and smoking status. Data concerning smoking habits were obtained by interviewing the subjects in association with the clinical examination and the subjects were categorized as non-smokers and smokers.

Overnight fasting venous blood samples were collected from 8:00 to 9:00 a.m. in plain, EDTA added tubes. The samples were analyzed immediately. Glucose and glycosylated hemoglobin (HbA1c) were analyzed by routine laboratory methods.

Periodontal Status

Diagnosis and classification of periodontal disease were made on the basis of clinical parameters and consisted of clinical examination, medical and dental history and assessment

of clinical attachment loss (CAL). CAL was measured by simple probing and identifying the cemento– enamel junction and measuring the distance to the base of the pocket and measurement of CAL were recorded at 4 points around each tooth.

The periodontal status of each subject was based on the amount of clinical attachment loss [46]. Patients found to exhibit no signs of periodontal disease as determined by the absence of CAL ≤ 3mm were considered healthy and the patients exhibiting of CAL ≥ 4mm were considered with chronic periodontitis.

Determination of the groups

191 elderly were classified into four groups: healthy control group (*n*=56), chronic periodontitis (CP) group (*n*=79), Type 2 diabetes mellitus (T2DM) group (*n*=19) and CP&T2DM group (*n*=37).

Sampling and DNA extraction

Elderly venous blood samples were obtained by venipuncture and collected into a EDTA containing vaccum tube. Total DNA was isolated from peripheral blood leukocytes, through the PureLink Kit - Invitrogen according to the manufacturer's instructions.

Determination of polymorphisms

Polymorphism in the IL6 gene at position G -174C (rs1800795)

The following primer pair was used for PCR amplification of genomic DNA samples: 5`-TTGTCAAGACATGCCAAGTGCT-3` (forward) and 5`-GCCTC AGAGACATCTCCAGTCC-3` (reverse) [47]. Amplification reactions were carried out with 100 ng/µL genomic DNA in a total volume of 50 µL, containing 10mM Tris-HCl (pH 8.3),

50mM KCl, 1 µM of each primer, 200 µM of each dATP, dCTP, dGTP and dTTP, 1.5mM $MgCl_2$, and 2.5U Taq DNA polymerase (Invitrogen). The reaction was incubated for 5 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C, and a final extension at 72°C for 5 min. After amplification, 10 µL of the PCR product was analyzed by agarose gel electrophoresis (1%). Subsequently the gel was stained with Syber-safe (Invitrogen) and the newly synthesized fragments were visualized under ultraviolet light. The size of the PCR amplified product was estimated from the electrophoretic migration of the product relative to the marker 100 bp DNA Ladder (Invitrogen). The products were digested with 1U of NlaIII (5'CATG 3') at 37°C Overnigth to detect allele G (13 bp +227 bp + 59 bp) and allele C (13 bp +118 bp +109 bp + 59 bp). The visualization of the digestion product is made in agarose gel electrophoresis in 2% stained with Syber-safe (Invitrogen).

Polymorphism in the TNFα gene at position G -308A (rs1800629)

The oligonucleotides 5`- AGG CAA TAG GTT TTG AGG GCC AT-3'and 5'-TCC TCC CTG CTC CGA TTC CG-3' were used as primers. Amplifications reactions were performed with 100 ng/µL genomic DNA in a total volume of 50 µL, containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 1 µM of each primer, 200 µM each dATP, dCTP, dGTP and dTTP, 1.5mM $MgCl_2$, and 2.5U Taq DNA polymerase (Invitrogen). The reaction was incubated for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, and a final extension at 72°C for 5 min. After amplification, 10 µl of the PCR product is analyzed by agarose gel electrophoresis (1%). Subsequently the gel is stained with Syber-safe (Invitrogen) and the newly synthesized fragments are visualized under ultraviolet light. The size of the PCR amplified product will be estimated from the electrophoretic migration of the product relative to the marker 100 bp DNA Ladder (Invitrogen). The products were digested with 1U per 25 µl reaction of NcoI (5'C↓CATGG 3') at 37°C

Overnight to detect allele G (87 bp +20 bp) and allele A (107 bp). The visualization of the digestion product is made in agarose gel electrophoresis in 2% stained with Syber-safe (Invitrogen).

Statistical analysis

Statistical analysis of data was performed with SPSS package versus 17.0 (SPSS Inc., Chicago, IL, USA). Subjects characteristics were expressed as means, standard deviation (±SD) and percents (Table 1). Differences in distributions of genotypes as well as frequencies of alleles for each polymorphism were analyzed by Fisher exact tests and Chi-square test among four groups. The association between T2DM group, CP group and genotype/allele was calculated as the odds ratio (OR) [95% confidence intervals (CIs)]. The differences of genotypes were further analyzed through logistic regression models with adjustment for age, gender and smoking. Hardy-Weinberg equilibrium was tested in each group by a chi-square test. P-value <0.05 were considered as statistically significant.

For the successive statistical analyses, genotypes at -174G/C locus were grouped as "C+" (CC and CG) and "C-"(GG) carriers.

Results

A description of patient characteristics is provided in Table 1. The mean age of patients was 67.8 years (SD=5.28). The male/female was 79/112 and 60.7% of the patients were considered no smokers. A significant association was found in gender and smoking between the patients and control group, with an increased presence of the male gender in the CP group (p=0.002) and smoking in the same group (p=0.005).

The results of genotypic and allelic frequencies of the locus *IL6-174* are presented in Table 2. The genotypes at two genes were in Hardy–Weinberg equilibrium (each *P*-value >0.05). A significant association was found in the locus *IL6*-174 for both genotypes distribution and allele frequencies among the four groups (*P*= 0.045 and 0.013 respectively). The C/C genotype frequency in CP (5.1%) was lower than the T2DM or T2DM & CP (15.8% or 10.8%), and control group shared the lowest frequency (3.6%). There was a significant difference in the allele frequency (p=0.0138) among the groups. Higher frequency for the allele G was to CP group (80.4%) and it was observed a higher proportion of the C allele for T2DM group (44.7%).

Allele G of the *IL6*-174 polymorphism was carried by 93.2% (178/191) of the elderly; of these 56% (107/191) were heterozygous 28% (30/107) belonged to the healthy group, 48.5% (52/107) to the CP group and only 5% (5/107) to the T2DM group. The frequency of allele G in the elderly studied was 74.6% and of allele C was 25.4%, respectively.

In detail, the multiple comparisons (Table 2) between T2DM and CP groups versus control group showed *P*-value significant and OR values in C-allele frequency of *IL6-174* (*P*=0,037, OR=2.429, 95% CI: 0.125-5.241 and P=0.001, OR=0.097, 95% CI: 0,041-0.228, respectively). However, in other multiple comparisons (GG vs CC+GC) in the same model,

no significant P-value was observed. In addition, in another model (CC vs GC+GG), the comparison among the four groups did not observe significant P-value (data not shown).

No significant differences were found for the *TNFα* genotype distribution or the alleles frequencies (Table 2). The genotype GG at position -308 was present in more than half of the patients and the rare allele A showed very low in the four groups.

According to the results, the C-allele of IL6 gene might be a protective factor of CP, which can be explained in the further logistic regression analysis with adjustment for age, gender and smoking (Table 3, *P*= 0.02, OR= 0.468, 95% CI: 0.243-0.904) (Table 3).

The table 3 shows a logistic regression analysis with significant P-value indicating that variables as female gender may be a protective factor for CP (P<0.01, OR= 0.293, 95% CI:0.142-0.584) and genotype may risk factor T2DM (P = 0.02, OR= 2.254, 95% CI: 1.161-4.375).

Discussion

Owing to the role of IL-6 and TNF-α as an inflammatory mediator, it is may modulate the predisposition to a number of inflammatory diseases such as atherosclerosis, rheumatoid arthritis and periodontitis [48, 31, 49]. So far, little information is available in the literatures about the association among *IL6* and *TNFα* promoter polymorphisms, diabetes and periodontitis, especially in the elderly population. In the present cross-sectional study, it was evaluated the possible role of polymorphisms in the *IL6*-174 and *TNFα*-308 genes on the risk of periodontitis in elderly with diabetes and elderly without diabetes.

In this study, an increased prevalence of the GG genotype was observed for the *IL6* polymorphism in non-diabetics elderly with periodontitis, suggesting that elderly harboring the GG genotype may be more susceptible to developing periodontitis. This is consistent with other studies showing increased IL6 GG prevalence in Caucasian subjects with aggressive periodontitis [50, 51] and in Brazilian individuals with chronic periodontitis [31, 43]. High frequency population-specific alleles are particularly useful for mapping genes responsible for disease susceptibility [52].

The findings of this study revealed that carriers of the C allele were significantly less affected by periodontal disease (P= 0.001, OR= 0.097, 95% CI: 0.041-0.228). Thus the results of this study also strengthen the statement referenced by Trevilatto et al. (2003)[31] and Costa et al. (2010)[43] that the presence of the C allele may represent a protective function as it may reduce IL-6 production. It is possible because the polymorphism at position -174 is located in a negative regulatory domain between -225 and -164 [48] having a negative regulatory effect on gene expression [53].

The low frequency of the CC genotype possibly is related to the low frequency of allele C in the population studied (25.4%). This low frequency is smaller than that of 41% found by Olomolaiye et al. (1998)[47] and 40.3% encountered by Fishman et al. (1998)[48] in

healthy Caucasian populations. However, Trevilatto et al. (2003)[31] reported similar findings in another Brazilian population (29.8%). The presence of the C allele therefore would result in a lower *IL6* expression after a given inflammatory stimulus compared with the G/G genotype (Fishman et al. 1998)[48], suggesting that this genotype confers a protective influence against the development of the disease, this can be observed as the C-allele frequency of *IL6*-174 in CP group was lower than that in the control group in this analysis.

In relation to the diabetic group, there was a higher significant prevalence of the CC genotype and C allele ($P < 0.05$). It suggested that the *IL6*-174 polymorphism could be considered a genetic marker able to discriminate two diabetic populations with different sensitivity toward IL-6. The presence of C allele defines a group of diabetic subjects in which insulin action is "IL-6-sensitive". Testa et al. (2006)[54] found C+ carries subjects have an insulin resistance "IL-6-sensitive", while C- carrier do not. This "insensitivity" to the effect of IL-6 levels in C- diabetic carriers suggests that in these subjects insulin resistance may depend on other factors, may be not linked to inflammation. The underlying mechanisms are not known. Fishman et al. (1998)[48] reported that C+ carries have a tight *IL6* gene expression regulation as the presence of cytosine in the position-174 creates a possible inhibitor-transcription-factor-biding-site.

Despite numerous reports on the role of TNF-α in the pathogenesis of periodontal disease [55, 56], in this study, there was no significant difference between genotypes and alleles of *TNFα* in the groups (Table 2). The A allele carriage rate for the *TNFα* polymorphism among the periodontitis and diabetic patients did not differ from that of the control group. Other studies that reported that genetic polymorphism in the *TNFα* gene at position -308 could not identify any genetic factor in periodontitis [41, 42, 57, 58]. As IL-6 is a multifunctional cytokine that regulates immune responses and its effects overlap those of IL-1 and tumor

necrosis factor (TNF) [31]. Given this suggested further studies to try to clarify or relationships which may exist between IL-6 and TNF-α in periodontal disease.

It has been a well-established consensus that diabetic patients are at significantly increased risk for periodontal complications [59-61]. It is suggested that diabetes and genetic factors put certain individuals at relatively higher risk for increased severity of periodontitis [39, 18]. Guzman et al. (2003)[62] evaluated periodontitis in diabetics with different *IL1* genotypes. Perez et al. (2004)[24] investigated the association between *TNFα*-308 in Chilean patients with aggressive periodontitis and/or T1DM. Struch et al (2008)[63] assessed the association between the *IL1* genotype and periodontitis in a diabetic and non-diabetic subjects. Xiao et al (2009)[19] found association among *IL6*-572 gene polymorphism, type 2 diabetes mellitus and periodontitis in a Chinese population. However, this study failed to find an association between *IL6*-174 polymorphism and elderly diabetic patients with periodontal disease (p=0.681, OR= 1.169, 95% CI:0.556-2.458).

A limitation of this study is that plasma IL-6 levels were not measured in this Brazilian elderly population. Further studies are required to interpret the results.

Although not demonstrated the mutual relationship between type 2 diabetes and genetic polymorphism of *IL6* with CP, it is concluded that more research is needed to better understand the comprehensive cause-effect relationships between diabetes, chronic periodontitis and genetic polymorphisms.

References

1. OGAWA H, YOSHIHARA A, HIROTOMI T, ANDO Y, MIYAZAKI H. Risk factors for periodontal disease progression among elderly people. J Clin Periodontol 2002; 29: 592-597.

2. LOCKER D, SLADE GD, MURRAY H. Epidemiology of periodontal disease among older adults: a review. Periodontology 2000 1998; 16: 16–33.

3. AINAMO J, SARKKI L, KYHALAMPI ML, PALOLAMPI L, PHRTO O. The frequency of periodontal extractions in Finland. Community Dental Health 1984; 1: 165– 172.

4. KAY EJ, BLINKHORN AS. The reasons underlying the extraction of teeth in Scotland. British Dental Journal 1986; 160: 287–290.

5. NIESSEN LC, WEYANT RJ. Causes of tooth loss in a veteran population. Journal of Public Health Dentistry 1989; 49: 19–23.

6. CHAUNCEY HH. Principle cause of tooth extraction in a sample of US male adults. Caries Research 1989; 23: 200–205.

7. REICH E, HILLER KA. Reasons for tooth extraction in the western states of Germany. Community Dentistry and Oral Epidemiology 1993; 21: 379–383.

8. BECK JD, CUSMANO L, GREENE-HELMS W, KOCH GG, OFFENBACHER S. A 5-year study of attachment loss in community- dwelling older adults: incidence density. Journal of Periodontal Research 1997; 32: 506–515.

9. SLADE GD, SPENCER AJ. Periodontal attachment loss among adults aged 60 in South Australia. Community Dentistry and Oral Epidemiology 1995; 23: 237–242.

10. BROWN LJ, BRUNELLE JA, KINGMAN A. Periodontal disease progression among the elderly 597 Periodontal status in the United States. 1988–91: Prevalence, extent, and demographic variation. Journal of Dental Research 1996; 75 (Spec Iss): 672–683.

11. ARMITAGE GC, WU YF, WANG HY ET AL. Low prevalence of a periodontitis-associated interleukin-1 composite genotyge in individuals of Chinese heritage. J Periodontol; 2000; 71: 164–171.

12. DAVID W, PAQUETTE NB, TIMOTHY C. Cardiovascular disease, inflammation, and periodontal infection. Periodontol 2000 2000 2007; 44: 113–126.

13. YOSHIE H, KOBAYASHI T, TAI H ET AL. The role of genetic polymorphisms in periodontitis. Periodontol 2000 2000 2007; 43: 102–132.

14. JETTE AM, FELDMAN HA, TENNSTEDT SL. Tobacco use: a modifiable risk factor for dental disease among the elderly. American Journal of Public Health 1993; 83: 1271–1276.

15. LOCKER D, LEAKE JL. Periodontal attachment loss in independently living older adults in Ontario, Canada. Journal of Public Health Dentistry 1993; 53: 6–11.

16. GENCO RJ. Current view of risk factors for periodontal diseases. Journal of Periodontology 1996; 67: 1041–1049.

17. PAGE RC. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. Annales of Periodontology 1998; 3: 108–120.

18. KORNMAN KS, CRANE A, WANG H-Y, DI GIOVINE FS, NEWMAN MG, PIRK FW WILSON JR. TG, HIGGINBOTTOM FL, DUFF GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997; 24: 72-77.

19. XIAO LM, YAN YX, XIE CJ, FAN WH, XUAN DY, WANG CX, CHEN L, SUN SY, XIE BY, ZHANG JC. Association among interleukin-6 gene polymorphism, diabetes and periodontitit in a Chinise population. Oral Diseases 2009; 15: 547-553.

20. TAYLOR G. Bi-directional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Annals of Periodontology 2001; 6: 99–112.

21. MEALEY BL, OATES TW. Diabetes mellitus and periodontal diseases. Journal of Periodontology 2006; 77: 1289–1303.

22. HOLLA LI, FASSMANN A, STEJSKALOVA A. Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. J Periodontol 2004; 75: 30–36.

23. ILLIG T, BONGARDT F, SCHOPFER A. Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. J Clin Endocrinol Metab 2004; 89: 5053–5058.

24. PEREZ C, GONZALEZ FE, PAVEZ V ET AL. The -308 polymorphism in the promoter region of the tumor necrosis factor-alpha(TNF-a) gene and ex vivo lipopolysaccharideinduced TNF-a expression in patients with aggressive periodontitis and ⁄ or type 1 diabetes mellitus. Eur Cytokine Netw 2004; 15: 364–370.

25. BRETT PM, ZYGOGIANNI P, GRIFFITHS GS ET AL. Functional gene polymorphisms in aggressive and chronic periodontitis. J Dent Res 2005; 84: 1149–1153.

26. HAMID YH, ROSE CS, URHAMMER SA ET AL. Varistions of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia 2005; 48: 251–260.

27. TAN WC, TAY FB, LIM LP. Diabetes as a risk factor for periodontal disease: current status and future considerations. Ann Acad Med Singap 2006; 35: 571–581.

28. DUARTE PM, NETO JBC, CASATI MZ ET AL. Diabetes modulates gene expression in the gingival tissues of patients with chronic periodontitis. Oral Dis 2007; 13: 594–599.

29. KORNMAN KS, DI GIOVINE FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. Annals of Periodontology 1998; 3: 327– 338.

30. GALBRAITH GMP, HENDLEY TM, SANDERS JJ, PALESCH Y, PANDEY JP. Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. Journal of Clinical Periodontology, vol. 26, no. 11, pp. 705–709, 1999.

31. TREVILATTO PC, SCAREL-CAMINAGA RM, DE BRITO RB JR, DE SOUZA AP, LINE SRP. Polymorphism at the position -174 of IL-6 is aasociated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. J Clin Periodontol 2003; 30: 438-442.

32. SCHULZ S, MACHULLA HKG, ALTERMANN W, ET AL. Genetic markers of tumour necrosis factor α in aggressive and chronic periodontitis. Journal of Clinical Periodontology 2008; 35: 493–500.

33. LOTZ M, JIRIK F, KABOURIDIS P, TSOUKAS C, HIRANO T, KISHIMOTO T, CARSON DA. B cell stimulating factor 2/interleukin- 6 is a costimulant for human thymocytes and IL-6 polymorphism and chronic periodontitis 441 T lymphocytes. Journal of Experimental Medicine 1988; 167: 1253–1258.

34. REVEL M. Host defence against infections of inflammations: role of the multifunctional IL-6/IFN-beta2 cytokine. Experientia 1989; 45: 549–557.

35. ISHIMI Y, MIYAURA C, JIN CH, AKATSU T, ABE E, NAKAMURA Y, YAMAGUCHI A, YOSHIKI Y, MATSUDA T, HIRANO T, KISHIMOTO T, SUDA T. IL-6 is produced by osteoblasts and induces bone resorption. Journal of Immunology 1990; 145: 3297–3303.

36. HUGHES FJ, HOWELLS GL. Interleukin-6 inhibits bone formation in vitro. Bone Miner 1993; 21:21–8.

37. BABEL N, CHEREPNEV G, BABEL D, ET AL. Analysis of tumor necrosis factor-β, transforming growth factor-β, interleukin- 10, IL-6, and interferon-γ gene polymorphisms in patients with chronic periodontitis. Journal of Periodontology 2006; 77: 1978–1983.

38. ALEXANDER MB, DAMOULIS PD. The role of cytokines n the pathogenesis of periodontal disease. Curr Opin Periodontol 1994; 2: 39-53.

39. OFFENBACHER S. Periodontal diseases: pathogenesis. Ann Periodontol 1996; 1: 821-878.

40. OKADA H, MURAKAMI S. Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med 1998; 9: 248-266.

41. FASSMANN A, IZAKOVICOVA HOLLA L, BUCKOVA D ET AL. Polymorphisms in the +252 (A/G) lymphotoxinalpha and the -308 (A/G) tumor necrosis factoalpha genes and susceptibility to chronic periodontitis in a Czech population. J Periodont Res 2003; 38: 394–399.

42. FOLWACZNY M, GLAS J, TOROK H-P, FRICKE K, FOLWACZNY C. Lack of association between the TNFa G -308 A promoter polymorphism and periodontal disease. Journal of Clinical Periodontology 2004; 31: 449-453.

43. COSTA AM, GUIMARÃES MCM, DE SOUZA ER, NÓBREGA OT, BEZERRA ACB. Interleukin-6 (G-174C) and tumour necrosis factor-alpha (G-308A) gene polymorphisms in geriatric patients with chronic periodontitis. Gerodontology 2010; 27: 70-75.

44. BARBETTA PA. Estatística aplicada às ciências sociais, 7ª ed. Florianópolis: UFSC, 2007.

45. SPIRDUSO WW. Dimensões físicas do envelhecimento. Barueri: Manole, 2005.

46. ARMITAGE GC. Development of a classification system for periodontal disease and conditions. Ann Periodontol 1999; 4: 1–6.

47. OLOMOLAIYE O, WOOD NA, BIDWELL JL. A novel NlaIII polymorphism in the human IL-6 promoter. Eur J Immunogenet 1998; 25: 267.

48. FISHMAN D, FAULDS G, JEFFEY R, MOHAMED-ALI V, YUDKIN JS, HUMPHRIES S, WOO P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. Journal of Clinical Investigation 1998; 102: 1369–1376.

49. FIFE MS, OGILVIE EM, KELBERMAN D, SAMUEL J, GUTIERREZ A, HUMPHRIES SE, WOO P. Novel IL-6 haplotypes and disease association. Genes and Immunity 2005; 6: 367–370.

50. TERVONEN T, RAUNIO T, KNUUTTILA M, KARTTUNEN R. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. Journal of Clinical Periodontology 2007; 34: 377–383.

51. NIBALI L, GRIFFITHS GS, DONOS N, PARKAR M, D'AIUTO F, TONETTI MS, BRETT PM. Association between interleukin-6 promoter haplotypes and aggressive periodontitis. Journal of Clinical Periodontology 2008; 35: 193–198.

52. STEPHENS JC, SCHNEIDER JA, TANGUAY DA ET AL. Haplotype variation and linkage disequilibriun in 313 human genes. Science 2001; 293: 489–493.

53. RAY A, LA FORGE KS, SEHGAL PB. On the mechanism for efficient repression of the interleukin-6 promoter by glucocorticoids: enhancer, TATA box, and RNA start site (Inr motif) occlusion. Molecular and Cellular Biology 1990; 10: 5736–5746.

54. TESTA R, OLIVIERI F, BONFIGLI AR, SIROLLA C, BOEMI M, MARCHEGIANI F, MARRA M, CENERELLI S, ANTONICELLI R, DOLCI A, PAOLISSO G, FRANCESCHI C. Interleukin-6–174 G > C polymorphism affects the association between IL-6 plasma levels and insulin resistance in type 2 diabetic patients. Diabetes Research and Clinical Practice 2006; 71: 299–305

55. GÓRSKA R, GREGOREK H, KOWALSKI J ET AL. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. J Clin Periodontol 2003; 30: 1046–1052.

56. GALBRAITH GM, STEED RB, SANDERS JJ ET AL. Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype. J Periodontol 1998; 69: 428–433.

57. CRAANDIJK J, VAN KRUGTEN MV, VERWEIJ CL ET AL. Tumor necrosis factor-a gene polymorphisms in relation to periodontitis. J Clin Periodontol 2002; 29: 28–34.

58. SAKELLARI D, KATSARES V, GEORGIADOU M ET AL. No correlation of five gene olymorphisms with periodontal conditions in a Greek population. J Clin Periodontol 2006; 33: 765–770.

59. LALLA E. Periodontal infections and diabetes mellitus: when will the puzzle be complete? J Clin Periodontol 2007; 34: 913–916.

60. SCULLY C. The influence of systemic diseases on oral health care in older adults. J Am Dent Assoc 2007; 138 (Suppl. 1): 7S–14S.

61. SKAMAGAS M, BREEN TL, LEROITH D. Update on diabetes mellitus: prevention, treatment, and association with oral diseases. Oral Dis 2008; 14: 105–114.

62. GUZMAN S, KARIMA M, WANG HY. Association between interleukin-1 genotype and periodontal disease in a diabetic population. J Periodontol 2003; 74: 1183–1190.

63. STRUCH F, DAU M, SCHWAHN C, ET AL. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the study of health in Pomeranis (SHIP). J Periodontol 2008; 79: 501–507.

Table 1. Baseline clinical parameters of the subject population (n= 191)

	Control (n=56)	*T2DM (n=19)*	*CP (n=79)*	*CP+T2DM (n=37)*	*p Value*
Gender *n* (%)					
Male	13(23.2)	5(26.3)	45(57.0)	16(43.2)	0.002
Female	43(76.8)	14(73.7)	34(43.0)	21(56.8)	
Age *n* (%)					
60-64 years	21(37.5)	06(31.6)	24(30.3)	12(32.4)	0.490
65-74 years	29(51.8)	10(52.6)	54(57.0)	20(54.1)	
≥75 years	06(10.7)	03(15.8)	10(12.7)	05(13.5)	
(mean± s.d)	67.32±5.03	68.47±5.91	67.99±5.46	67.97±5.12	
Smoking *n* (%)					
No-smoking	44(78.6)	11(57.9)	40(50.6)	21(56.8)	0.005
Smoking	12(21.4)	8(42.1)	39(49.4)	16(43.2)	

Table 2. The genotype distribution and the allele frequencies for the -G174C of interleukin-6 and –G308A of tumor necrosis factor-alfa in a Brazilian elderly population

	Control (n=56)	*T2DM (n=19)*	*CP (n=79)*	*CP+T2DM (n=37)*	*p Value*
Genotypes					
IL6-174					
G/G	30 (53.6)	05(26.3)	52(65.8)	20(54.1)	0.0450
C/C	02(03.6)	03(15.8)	04(5.1)	04(10.8)	
G/C	24(42.9)	11(57.9)	23(29.1)	13(35.1)	
TNFα-308					
G/G	40(71.4)	15(78.9)	48(60.8)	23(62.2)	0.1830
A/A	07(12.5)	01(05.3)	11(13.9)	06(16.2)	
G/A	09(16.1)	03(15.8)	20(25.3)	8(21.6)	
Alleles					
IL6					
G	84 (75.0)	21 (55.3)	127(80.4)	53 (71.6)	0.0138
C	28 (25.0)	17(44.7)	31 (19.6)	21(28.4)	
TNFα					
G	89 (79.4)	33 (86.84)	116 (73.41)	54 (72.97)	0.2460
A	23 (20.6)	5 (13.15)	42 (26.58)	20 (27.02)	

-174*IL6*	T2DM *vs* Control	CP *vs* Control	CP+T2DM *vs* Control
GG*vs*GC+CC			
P-value	0.1893	0.9952	0.3372
OR(95%CI)	5.06(0.77-32.98)	1.44 (0.25-8.15)	3.27 (0.57-18.87)
C-allele *vs* G-allele			
P-value	0.037	0.001	0.732
OR(95%CI)	2.429 (0.125-5.241)	0.097 (0.041-0.228)	1.189 (0.613-2.305)

Table 3 Regression analyses for associated risk factors of CP and DM in a Brazilians elderly

	T2DM vs control	CP vs control	CP&T2DM vs Control
Gender			
P-value	0.201	0.000	0.974
OR(95% CI)	1.566 (0.788- 3.112)	0.293(0.147-0.584)	1.013(0.474-2.165)
Age			
P-value	0.963	0.960	0.951
OR(95% CI)	1.017(0.497-2.082)	0.982(0.490-1.971)	0.975(0.435-2.187)
Smoking			
P-value	0.182	0.057	0.554
OR(95% CI)	1.593(0.804-3.156)	1.937(0.980-3.827)	1.259(0.587-2.701)
TNFα			
P-value	0.915	0.623	0.553
OR(95% CI)	0.948(0.356-2.525)	1.278(0.481-3.401)	1.368(0.486-3.852)
IL6			
P-value	0.02	0.02	0.681
OR(95% CI)	2.254(1.161-4.375)	0.468(0.243-0.904)	1.169(0.556-2.458)

OR, odds ratio; CI, confidence interval; DM, diabetes mellitus; CP, chronic periodontits; TNF, tumour necrosis factor; IL, interleukin.

Gender (dichotomous, male=1 and female=2), age (continuous), smoking (dichotomous, no=1 and yes=2), TNFα (dichotomous GG=1 and AA+GA=2), IL6 (dichotomous GG=1 and CC+GC=2). *P*-values < 0.05 were considered significant

5 CONCLUSÃO

Com base na metodologia utilizada e a partir dos resultados obtidos, pode-se concluir que:

1. Houve significativa associação do polimorfismo no gene da Interleucina 6, gênero masculino e hábito de fumar com a DP entre os idosos estudados.

2. Não houve associação estatisticamente significante entre o polimorfismo G-308A no gene do Fator de necrose Tumoral alfa e os idosos estudados.

3. Houve um significativo aumento da frequência do alelo C no grupo controle do gene da *IL6-174*, sugerindo um fator protetor à periodontite crônica.

4. Foi identificado como fator de risco a diabetes os genótipos CC+CG do gene *IL6*.

- Não houve associação estatisticamente significante entre os polimorfismos nos genes da interleucina 6 e *TNFA* nos idosos portadores de ambas as doenças: diabetes mellitus e periodontite crônica.

REFERÊNCIAS

1. Wong LLR, Carvalho JÁ. O rápido processo de envelhecimento populacional do Brasil: sérios desafios para as políticas públicas. Revista Brasileira de Estudos de População 2006; 26 (1): 5-2.

2. Carvalho JÁ, Rodrigues-Wong LLA. Transição da estrutura etária da população brasileira na primeira metade do século XXI. Cadernos de Saúde Pública, Rio de Janeiro 2008; 24(3): 597-605.

3. Ogawa H, Yoshihara A, Hirotomi T, Ando Y, Miyazaki H. Risk factors for periodontal disease progression among elderly people. J Clin Periodontol 2002; 29: 592–597.

4. Slade GD, Spencer AJ. Periodontal attachment loss among adults aged 60 π in South Australia. Community Dentistry and Oral Epidemiology 1995; 23: 237–242.

5. Brown LJ, Brunelle, JA, Kingman A. Periodontal status in the United States. 1988–91: Prevalence, extent, and demographic variation. Journal of Dental Research (Spec Iss). 1996; 75: 672–683.

6. Ainamo J, Sarkki, L, Kyhalampi ML, Palolampi L, Phrto O. The frequency of periodontal extractions in Finland.Community Dental Health. 1984; 1:165–172.

7. Kay EJ, Blinkhorn AS. The reasons underlying the extraction of teeth in Scotland. British Dental Journal. 1986; 160: 287–290.

8. Bailit, HL, Braun R, Maryniuk GA, Camp P. Is periodontal disease the primary cause of tooth extraction in adults. Journal of American Dental Association. 1987; 114: 40–45.

9. Niessen LC, Weyant RJ. Causes of tooth loss in a veteran population. Journal of Public Health Dentistry. 1989; 49: 19–23.

10. Chauncey HH. Principle cause of tooth extraction in a sample of US male adults. Caries Research. 1989; 23: 200–205.

11. Reich E, Hiller KA. Reasons for tooth extraction in the western states of Germany. Community Dentistry and Oral Epidemiolog. 1993; 21: 379–383.

12. Beck JD, Slade GD. Epidemiology of periodontal diseases. Current Opinion in Periodontology. 1996; 3: 3–9.

13. Johnson T E. Factors contributing to dentists' extraction decisions in older adults. Special Care in Dentistry.1993; 13: 195–199.

14. Miyazaki H, Ohtani I, Abe N, Ansai T, Katoh Y, Sakao S, Takehara T, Shimada N, Pilot T. Periodontal conditions in older age cohorts aged 65 years and older in Japan, measured by CPITN and loss of attachment. Community Dental Health. 1995; 12: 216–220.

15. Bodet C, Chandad F, Grenier D. Porphyromonas gingivalisinduced inflammatory mediator profile in an ex vivo human whole blood model. Clin Exp Immunol. 2005; 143: 50-57.

16. Kahnberg KE, Lindhe J, Helldén L. Initial gingivitis induced by topical application of plaque extracts. A histometric study in dogs with normal gingivae. Journal of Periodontal Research. 1976; 11: 218-225.

17. Liljenberg B, Lindhe J, Berglundh T, Dahlén G. Some microbiological, histopathological and immunohistochemical characteristics of progressive periodontal disease. Journal of Clinical Periodontology. 1994; 21: 720-715.

18. Lindhe J. Tratado de periodontologia clínica e Implantodontia. Rio de Janeiro, Editora Guanabara.1999.

19. Offenbacher S, Barros SP, Beck JD. “Rethinking periodontal inflammation,” Journal of Periodontology. 2008; 79(8): 1577–1584.

20. Brett PM, Griffiths GS, Tomaz M, Parkar M, D’Aiuto F, Tonetti M. "Functional Gene Polymorphisms in Aggressive and Chronic Periodontitis." J DENT RES. 2005; 84: 1149.

21. Shapira L, Wilensky A, Kinane DF. " Effect of genetic variability on the inflammatory response to periodontal infection”. J. Clin. Periodontol. 2005; 32(6): 72-86.

22. Laine ML, Crielaard W. "Gene Polymorphisms in Chronic Periodontitis." International Journal of Dentistry. 2010; 22.

23. Tabor, H. K., Risch, N. J., and Myers, R. M., “Candidate-gene approaches for studying complex genetic traits: practical considerations,” Nature Reviews Genetics. 2002; 3(5): 391–397.

24 Trevilatto PC, Brito RB Jr, Souza AP, Line SR. "Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population." J Clin Periodontol. 2003; 30: 438-42.

25. Moreira PR, Gomez RS, Gollob K.J, Dutra WO. "IL10 and TNFA Polymorphisms in Brazil." The Open Dentistry Journal. 2009; 3: 184-190.

26. World Health Organization. W. H. O. "What is the burden of oral disease?" from www.who.int/oral_health/disease_burden/global/en/print.html, 2000.

27. World Health Organization. The WHO Global Oral Health Data Bank. Geneva: World Health Organization. 2003.

28. Brasil, Ministério da Saúde. A política nacional de saúde bucal do Brasil: Registro de uma conquista histórica. Brasília 2004.

29. Brasil, Ministério da Saúde. Levantamento SB Brasil 2010. 2011. Disponível em: <http://www.idisa.org.br/img/File/SAUDE%20BUCAL--NotaParaImprensa-28dez2010%20(2).pdf> Acesso em: 03 nov 2011.

30. Sallum, A. W. Fatores modificadores do processo saúde-doença periodontal. Periodontia: Ciência e Clínica. A. Médica. São Paulo. 2001: 221-231.

31. Marsh PD, Bradshaw DJ. "Dental plaque as a biofilm." Journal of Industrial Microbiology and Biotechnology. 1995; 15(3): 169-175.

32. Darveau RP, Tanner A, Page RC. "The microbial challenge in periodontitis." Periodontology 2000. 2007; 14(1): 12-32.

33. Chung RM, G JT, Lamster IB. "Interleukin-8 and β-glucuronidase in gingival crevicular fluid." Journal of Clinical Periodontology. 1997; 24: 146-152.

34. Anne-Laure E, Igondjo-Tchen S, Karim S, Pellat B, Godeau G, Gogly B. "Are Cytokines Linked to Collagen Breakdown During Periodontal Disease Progression?" Journal of Periodontology 74: 196-201, 2003.

35. Gore EA, Sanders JJ, Pandey JP, Palesch Y, Galbraith GM." Interleukin-1beta_3953 allele 2: association with disease status in adult periodontitis." J. Clin. Periodontol. 1998; 25: 781-785.

36. American Academy of periodontology, Tobacco use and the periodontal patient. J. Periodontol. 1996; 1: 51-56.

37. Dinsdale CR. Subgingival temperature in smokers and non-smokers with periodontal disease. J. Clin. Periodontol. 1997; 24(10): 761-766.

38. Axelsson P. Relationship between smoking and dental status in 35, 50, 65 and 75 year-old individuals. J. Clin. Periodontol. 1998; 25: 297-305.

39. Croucher Ray MWS, Torres MC, Hughes F, Sheiham A . "The relationship between life-events and periodontitis A case-control study." Journal of Clinical Periodontology. 2005; 24(1): 39-43.

40. Janket SJ, Wightman A, Baird AE, Van Dyke TE, Jones JA. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. J Dent Res 2005;84:1154-1159.

41. Mealey BL. World Workshop in Clinical Periodontics. Periodontal implications: Medically compromised patients. Ann Periodontol 1996;1:256-321.

42. Papapanou PN. Periodontal diseases: Epidemiology Ann Periodontol 1996;1:1-36. Carranza FA, Newman MG. Periodontia Clínica. 10ª ed. Rio de Janeiro: Guanabara Koogan, 2007.

43. Michalowicz BS, Virag JG, Klump DG, Hinrichs JE, Segal NL ."Periodontal findings in adult twins." J Periodontology 2000. 1991; 62: 293-299.

44. Corey LA, N. W., Hofstede P, Schenkein HA. "Selfreported periodontal disease in a Virginia twin population." J. Periodontol. 1993; 64: 1205-1208.

45. Johson NW, G. G., Wilton JM, Maiden MF, Curtis MA, Gillett IR. "Detection of high-risk groups and individuals for periodontal diseases. Evidence for the existence of high-risk groups and individuals and approaches to their detection. ." J Clin Periodontol 1988; 15: 276-282.

46. Jenkins WMM, Kinane DF. "The high-risk group in periodontitis." Br Dent J. 1989; 167: 168-171.

47. Kinane DF, Hart TC. "Genes and gene polymorphisms associated with periodontal disease".Crit. Rev. Oral Biol. Med. 2003; 14: 430.

48. Sallum AW, Martins AG, Sallum EA. A doença periodontal e o surgimento de um novo paradigma. Periodontia Medica: uma abordagem integrada. São Paulo, Senac. 2004; 1: 22-39.

49. Greenstein G, Hart T. "A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis." J Periodontol. 2002; 73: 231-47.

50. Klein WT, A GT. "The polymorphism at position -174 of the IL-6 gene is not associated with inflammatory bowel disease. " Eur J Gastroenterol Hepatol. 2001; 13: 45-7.

51. Terry CT, LV, G. FR. "Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation." J Biol Chem. 2000; 24: 1813-4.

52. Hughes FJ, Howells G. " Interleukin-6 inhibits bone formation in vitro." Bone Miner. 1993; 21: 21-8.

53. Irwin CR, Myrillas T. "The role of IL-6 in the pathogenesis of periodontal disease." Oral Dis. 1998; 4: 43-7.

54. Okada H, Murakami S. "Cytokine expression in periodontal healthand disease." Crit Rev Oral Biol Med. 1998; 9: 248-66.

55. Mccauley L.K, Nohutch R. "Mediators of periodontal osseous destruction and remodeling. principles and implications for diagnosis and therapy." J Periodontol. 2002; 73: 1377-91.

56. D'Aiuto FPM, Andreou G, Brett PM, Ready D, Tonetti MS. " Periodontitis and atherogenesis: causal association or simple coincidence?" J Clin Periodontol. 2004; 31: 402-11.

57. Birkedal-Hansen H. "Role of cytokines and inflammatory mediators in tissue destruction." J Periodontal Res. 1993; 28: 500-10.

58. Rossomando EF, K J, Hadjimichael J. "Tumour necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans." Arch Oral Biol. 1990; 35: 431-4.

59. Ide M, Jagdev D, Coward PY, Crook M, Barcllay GR, Wilson RF. The short-term effects of treatment of chronic periodontitis on circulating levels of endotoxina, C-reactive protein, tumor necrosis factor-a and interleukin-6. J Peridontol. 2004; 75 (3): 420-428.

60. Genco R, Grossi S, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. J Periodontol. 2005; 76(11):2075-84.

61. Dufour A, Baran C, Langkamp HL, Piesco NP, Agarwal S. Regulation of differentiation of gingival fibroblast and periodontal ligament cells by rhIL-1 b and rhTNF-a. J Periodontal Res. 1993; 28: 566-8.

62. Galbraith GMP, S R, Sanders JJ, Pandey JP. "Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype." J Periodontol. 1998; 69: 428-433.

63. Craandijk J, V KM, Verweij CL, Van der Velden U, Loos BG. "Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis." J Clin Periodontol. 2002; 29: 28-34.

64. Xiao LM, Yan YX, Xie CJ, Fan WH, Xuan DY, Wang CX, Chen L, Sun SY, Xie BY, Zhang JC. Association among interleukin-6 gene polymorphism, diabetes and periodontitit in a Chinise population. Oral Diseases 2009; 15: 547-553.

65. Malecki MT. Genetics of type 2 diabetes mellitus. Diabetes Res Clin Pract 2005; 68(Suppl. 1): S10-S21.

66. Takeda, M., Ojima, M., Yoshioka, H., Inaba, H., Kogo, M., Shizukuishi, S., Nomura, M. & Amano, A. (2006) Relationship of serum advanced glycation end products with deterioration of periodontitis in type 2 diabetes patients. Journal of Periodontology 77, 15–20.

67. Tervonen, T., Raunio, T., Knuuttila, M. & Karttunen, R. (2007) Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. Journal of Clinical Periodontology 34, 377–383.

68. Taylor, G. (2001) Bi-directional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Annals of Periodontology 6, 99–112.

69. Mealey, B. L. & Oates, T. W. (2006) Diabetes mellitus and periodontal diseases. Journal of Periodontology 77, 1289–1303.

70. Tan WC, Tay FB, Lim LP (2006). Diabetes as a risk factor for periodontal disease: current status and future considerations. Ann Acad Med Singap 35: 571–581.

71. Duarte PM, Neto JBC, Casati MZ et al (2007). Diabetes modulates gene expression in the gingival tissues of patients with chronic periodontitis. Oral Dis 13: 594–599.

ANEXOS

ANEXO A - Parecer do Comitê de Ética em Pesquisa da UNOPAR

UNOPAR

Universidade Norte do Paraná
Pró-Reitoria de Pesquisa e Pós-Graduação
Coordenadoria de Pesquisa

Parecer referente ao projeto:

Protocolo: *0253/11*

Título: *"Estudo de associação entre polimorfismo de nucleotídeo único em genes candidatos para a doença periodontal em idosos."*

Responsável: *Regina Célia Poli-Frederico* (UNOPAR)

Categoria de projeto: *Pesquisa*

Cronograma: *set/11 – ago/12*

Parecer:

O projeto foi submetido à avaliação por consultores *ad hoc* e atende as necessidades do Curso e do Centro em que está vinculado, conforme pareceres dos respectivos dirigentes.

Os alunos envolvidos neste projeto deverão ser cadastrados na Coordenadoria de Pesquisa por meio dos formulários específicos disponíveis na Internet no site http://www2.unopar.br/pesquisa/pesquisa_formularios.htm

Referente ao item 8, a liberação dos materiais e equipamentos dependerá da aprovação do órgão competente. Para encaminhamento solicita-se o cronograma de atividades e desembolso.

O acompanhamento das atividades seguirá conforme previsto na Resolução CONSUN 020/2001.

O arquivo eletrônico do projeto deve ser enviado por e-mail para pesquisa@unopar.br

Qualquer alteração no projeto deve ser comunicada à Coordenadoria de Pesquisa.

As produções científicas e contribuições esperadas indicadas no projeto devem ser encaminhadas por meio do relatório final até a data de término previsto no cronograma.

Considerando a análise anterior o projeto está aprovado no mérito técnico/científico e cadastrado na Coordenadoria de Pesquisa com o número **PP/0208/11**.

Londrina, 01 de setembro de 2011.

Profª. Audrey de Souza Marquez
Diretora do Centro de Pesq. em Ciências da Saúde

Prof. Dr. Hélio N. Suguimoto
Pró-Reitor de Pesq. e Pós-Graduação

ANEXO B – Ficha de exame – Condições bucais

INFORMAÇÕES GERAIS

DATA: ___/___/_____
EXAMINADOR:_____
ANOTADOR:_____

Nº. IDENTIFICAÇÃO :_____

NOME:_____

IDADE:☐☐ **SEXO:** ☐ **1.MASC** ☐ **2.FEM**

CÁRIE DENTÁRIA E NECESSIDADE DE TRATAMENTO

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
																COROA
																RAIZ
																TRAT.
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	
																COROA
																RAIZ
																TRAT

EDENTULISMO

USO DE PRÓTESE

SUP. **INF.**

☐ ☐

NECESSIDADE DE PRÓTESE

SUP. **INF.**

☐ ☐

TEMPO DE USO

SUP: _____

INF:_____

CONDIÇÃO PERIODONTAL

CPI

SANGRAMENTO GENGIVAL

17/16 11 26/27

47/46 31 36/37

CÁLCULO DENTÁRIO

17/16 11 26/27

47/46 31 36/37

BOLSA PERIODONTAL

17/16 11 26/27

47/46 31 36/37

PIP

17/16 11 26/27

47/46 31 36/37