ANA PAULA RIBEIRO BRAOSI

Polimorfismos e Expressão dos Genes *IL1A*, *IL1B* e *IL1RN* e sua Associação com a Doença Renal Crônica e a Periodontite

CURITIBA
2008

ANA PAULA RIBEIRO BRAOSI

Polimorfismos e Expressão dos Genes *IL1A*, *IL1B* e *IL1RN* e sua Associação com a Doença Renal Crônica e a Periodontite

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde (PPGCS) do Centro de Ciências Biológicas e da Saúde (CCBS) da Pontifícia Universidade Católica do Paraná (PUCPR), como parte dos requisitos para a obtenção do título de Doutor em Ciências da Saúde, Área de Concentração Medicina e áreas afins.

Orientadora: Profª. Dra. Paula Cristina Trevilatto

CURITIBA
2008

DEDICATÓRIA

Dedico o trabalho especialmente aos meus pais, Renato e Rogéria (*in memoriam*), e aos meus irmãos Cláudia e Renato Júnior, pela grandessíssima força em toda a minha vida, em todos os momentos de paz e atribulações, em todas as situações.

Dedico também ao meu marido Leandro, o qual entendeu perfeitamente a importância da realização e finalização deste trabalho, apoiando-me e incentivado-me todos os dias em que estamos juntos.

Nesta oportunidade, peço perdão pelas minhas ausências e pelos momentos em que nossa alegria não foi completa.

Sem minha família, nada poderia ter sido realizado, nem nada teria tanto valor.

AGRADECIMENTOS

Especialmente a Deus, por permitir todos os momentos de minha vida e por presentear-me com a realização dos meus sonhos.

À Pontifícia Universidade Católica do Paraná (PUCPR), representada pelo seu Magnífico Reitor, **Prof. Dr. Clemente Ivo Juliatto**, instituição responsável por minha qualificação acadêmica e onde fui acolhida com apreço, inclusive possibilitando-me, pela concessão de bolsas de estudos, a completar minha sólida formação.

Ao Pró-Reitor de Graduação, Pesquisa e Pós Graduação, **Prof. Dr. Robert Carlisle Burnett**.

Ao Diretor de Pesquisa e Pós-Graduação, **Prof. Dr. Waldemiro Gremski,** pelo seu grande empenho e dedicação no desenvolvimento do Programa de Pós-Graduação em Ciências da Saúde.

Agradeço à minha brilhante orientadora **Profa. Dra. Paula Cristina Trevilatto,** Coordenadora de Pesquisa e Pós-Graduação da PUCPR, por tanta dedicação e entusiasmo na realização dos trabalhos e na formação de doutores competentes. Em quem me espelho e projeto para aperfeiçoar a cada dia meu trabalho. Muitíssimo obrigada!

Agradeço especialmente ao Coordenador do PPGCS, Prof. Dr. Roberto Pecoits-Filho, sempre muito atento e dedicado à formação de pessoal de nível superior.

Às secretárias do PPGCS da PUCPR, especialmente **Alcyone**, pela atenção e auxílio durante todo o curso.

Especialmente ao amigo e companheiro de doutorado **Dr. Cleber Machado de Souza**, o qual muitíssimo me ajudou na execução e finalização do trabalho.

A todos os amigos e colegas da pós-graduação da PUCPR, especialmente à minha grande amiga **Giovana Pecharki, Sônia Luczyszyn, Andréa Doetzer, Acir Dirschnabel, Luciana Kalinke, Cláudia Montes e Fabiano Alvim,** por todos os momentos que passamos juntos.

A todos os pesquisadores e funcionários do IBMP, que sempre nos ajudaram durante a execução de nossos experimentos.

À **Profa. Dra. Márcia Olandonski,** pela valiosa colaboração, realizando a análise estatística desse estudo, e pela competência e disponibilidade ao atender-me sempre que solicitei seu auxílio.

A todos os **professores do** PPGCS - Centro de Ciências Biológicas e da Saúde (CCBS) - e do PPGO – Programa de Pós Graduação em Odontologia da PUCPR, pelo conhecimento adquirido ao longo dos anos.

À Universidade Positivo, nas pessoas da **Professora Maria da Graça Kfouri Lopes**, coordenadora do Curso de Odontologia, e do Magnífico reitor **Dr. Oriovisto Guimarães**, instituição de ensino que me acolheu como docente no curso de graduação e pós-graduação em Odontologia, o que me proporciona grande alegria, conhecimento e experiência.

Agradeço muitíssimo aos meus amigos e colegas de disciplinas, **Dr. Allan Fernando Giovanini, Prof. Francisco Miguel Stroparo, Profa Lucienne Ullbrich e Profa. Sibele Pereira de Oliveira**, bem como à minha chefia na SMS Enfermeira **Márcia Bora** e Cirurgiã Dentista **Ivana Busatto**, por me darem apoio nos momentos em que mais precisei, possibilitando e compreendendo minhas ausências e afastamentos do trabalho.

“O seu tempo é limitado,

então não o gaste vivendo a vida de um outro alguém.

Não fique preso pelos dogmas, que é viver com os resultados da vida de outras pessoas.

Não deixe que o barulho da opinião dos outros cale a sua própria voz interior.

E o mais importante: tenha coragem de seguir o seu próprio coração e a sua intuição.

Eles de alguma maneira já sabem o que você realmente quer se tornar.

Todo o resto é secundário."

(Steve Jobs)

SUMÁRIO

SUMÁRIO

Resumo..... 9

Abstract..... 10

Introdução..... 12

Objetivos..... 17

Artigo..... 19

Conclusão..... 50

Referências..... 52

Anexos..... 59

RESUMO

A doença renal crônica (DRC) é uma doença progressiva, que cursa com a destruição dos nefros. A doença renal em estágio final, ou estágio 5, refere-se à fase em que os pacientes necessitam de terapia de substituição de suas funções renais, por diálise ou transplante. Pacientes com DRC apresentam diversas complicações, sendo a doença periodontal (DP) exacerbada nesses indivíduos. A DP é uma doença de natureza inflamatória crônica, iniciada por bactérias específicas que ativam mecanismos inflamatórios e geram a destruição dos tecidos periodontais. Embora as bactérias sejam essenciais para a iniciação da periodontite, há fatores modificadores que amplificam alguns mecanismos de progressão e severidade, como a presença de doenças sistêmicas e polimorfismos genéticos. Polimorfismos funcionais nos genes da IL-1 têm sido associados a diversas doenças imuno-inflamatórias. O objetivo deste trabalho foi investigar a associação entre polimorfismos nos genes do cluster da IL-1 [*IL1A*-889, *IL1B*-511, *IL1B*+3954 e *IL1*RN, íntron 2 (número variável de repetições em tandem – VNTR)] e a susceptibilidade à DRC e à DP. Também, o nível de transcritos gênicos foi comparado entre os grupos e de acordo com os genótipos dos quatro polimorfismos. Foram avaliados 246 indivíduos, com média de idade de 44,8 anos, divididos em: *grupo 1* (64 pacientes sem DRC e sem DP), *grupo 2* (58 pacientes sem DRC e com DP), *grupo 3* (52 pacientes com DRC e sem DP) e *grupo 4* (72 pacientes com DRC e com DP). O DNA genômico foi obtido a partir de células da mucosa bucal e os polimorfismos *IL1A*-889, *IL1B*-511, *IL1B*+3954 e *IL1*RN (íntron 2 - VNTR) foram estudados por reação em cadeia da polimerase (PCR) e pela técnica de análise de polimorfismos por tamanho de fragmento de restrição (RFLP), enquanto que os transcritos gênicos (obtidos a partir de tecido gengival) foram analisados por PCR em tempo real. O risco associado com genótipos, alelos e haplótipos foi calculado como *odds ratio* (OR) com intervalo de confiança de 95%. Possíveis diferenças na intensidade da expressão dos transcritos gênicos entre os grupos foram avaliadas por ANOVA. O alelo *IL1RN**1 foi associado a um risco cerca de 3 vezes maior para o desenvolvimento da DRC (OR 2,86 95% CI=1,1-7,4, *p*=0,045). Além disso, o alelo *IL1RN**2 foi associado à DP em pacientes com DRC (OR 3,53 95% CI=1,5-8,4, *p*=0,005), bem como o alelo *IL1B*+3954*T se associou à DRC em pacientes com DP (OR 1,96 95% CI=1,10-3,48, *p*=0,030). Níveis aumentados dos transcritos gênicos *IL1A*, *IL1B* e *IL1RN* foram observados em pacientes com DP, não mostrando diferenças de acordo com genótipos específicos. Concluiu-se que polimorfismos nos genes *IL1* associaram-se com a DRC e a DP. Maiores níveis de transcritos gênicos *IL1* refletiram a expressão aumentada de mediadores inflamatórios em sítios com DP. Parâmetros clínicos tradicionalmente usados na determinação do prognóstico de doenças inflamatórias complexas, como a DP e DRC, parecem estar, pelo menos em parte, na dependência de genótipos específicos.

ABSTRACT

Chronic kidney disease (CKD) is an inflammatory progressive illness characterized by the gradual and usually permanent loss of kidney function over time. The main causes are hypertension and diabetes mellitus. Patients with CKD present several complications, being the periodontal disease (PD) exacerbated in these individuals. Periodontitis is initiated by periodontal pathogens which, in turn, trigger the chronic inflammatory and immune responses that are thought to determine the clinical outcome of the disease. Although the bacteria is essential to the onset of PD, there are modifying factors which amplify mechanisms of severity and progression, such as systemic diseases and genetic polymorphisms. Therefore, the aim of this study was to investigate the association between the functional polymorphisms in the IL-1 cluster genes (*IL-1A*-889, *IL-1B*-511, *IL-1B*+3954 and *IL-1*RN, intron 2) and the susceptibility to CKD and PD. The study population consisted of 246 individuals, mean age 44.8 years, and was divided into: *group 1* (64 patients without CKD and without PD), *group 2* (58 patients without CKD and with PD), *group 3* (52 patients with CKD and without PD) and *group 4* (72 patients with CKD and with PD). Genomic DNA was obtained from cells of oral mucosa and the polymorphisms *IL1A*-889, *IL1B*-511, *IL1B*+3954 e *IL1*RN (intron 2) were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Interleukin-1 gene transcripts from gingival tissues were analyzed by real time-PCR technique. The risk associated with genotypes, alleles and haplotypes was calculated as the odds ratio (OR) with 95% confidence intervals (CI). To access possible differences in the intensity of mRNA expression among the groups was assessed by ANOVA (p<0.05). The results suggest that IL1RN*1 allele (the most frequent allele) was associated with a roughly 3-fold increased risk of CKD (OR 2.86 95% CI=1.1-7.4, p=0.045). The *IL1RN**2 allele was associated with PD in CKD patients (OR 3.53 95% CI=1.5-8.4, p=0.005), as well as *IL1B*+3954*T allele associated with CKD when patients with PD were analyzed (OR 1.96 95% CI=1.10-3.48, p=0.030). When real-time PCR was used to evaluate the expression of IL-1 genes, increased levels of transcripts of *IL1A*, *IL1B* and *IL1RN* genes were observed in PD patients, although with no statistically significant differences between groups without and with CKD. The present study concluded that there is an association between *IL1* gene polymorphisms and susceptibility to CKD and PD. Moreover, higher levels of *IL1* gene transcripts of the inflammatory mediators were found in sites with PD. Clinical parameters traditionally used to determine the outcome of inflammatory complex diseases, such as CKD and PD, seem to be, at least in part, dependent on specific genotypes.

INTRODUÇÃO

INTRODUÇÃO

A doença renal crônica (DRC) é um processo que expressa a perda da capacidade funcional dos nefros, independente de sua etiologia. Pode ser classificada em aguda, subaguda ou crônica, de acordo com seu início e com a possibilidade de retorno à normalidade das lesões estruturais (1). A DRC pode ser definida a partir de dois critérios: função e morfologia renal anormais (proteinúria) ou taxa de filtração glomerular (GFR) menor que 60 mL/min, calculada a partir de variáveis da fórmula *Modification of Diet in Renal Disease* (MDRD) utilizando as medidas de creatinina sérica (2). Pela GFR, a DRC pode ser classificada do estágio 1 ao estágio 5, de acordo com a GFR, sendo o estágio 5 um grau de doença avançado denominado de doença renal crônica em estágio final (ESRD), quando existe a necessidade da instalação de uma terapia de substituição que pode ser a diálise ou transplante renal (3, 4). Constitui um problema de saúde pública em muitos países (5). Segundo dados da Sociedade Brasileira de Nefrologia de 2008, existem cerca de 79 mil pacientes com DRC no Brasil, estando 36 mil (89,4%) em hemodiálise, e 8% novos casos surgindo ao ano (6). Pacientes em diálise apresentam maior prevalência de complicações, como doenças cardiovasculares, principal causa de morte nesses pacientes. Além disso, pacientes com DRC são mais suscetíveis à exacerbação de doenças infecciosas, como a doença periodontal (DP), o que pode contribuir para o aumento da morbidade e mortalidade nesses pacientes, como foco de inflamação (7-10).

A DP é uma doença infecciosa de natureza inflamatória crônica que leva à destruição patológica dos tecidos de suporte do dente e reabsorção óssea alveolar (11). Embora existam bactérias relacionadas com a iniciação da periodontite, o hospedeiro pode estar exposto a um desafio antigênico aumentado em sítios de doença periodontal destrutiva, acionando fatores modificadores que não causam a doença, mas amplificam alguns mecanismos de progressão e severidade, como o aumento dos níveis de citocinas pró-inflamatórias e ativação de anticorpos específicos em indivíduos suscetíveis (9). A presença de doenças sistêmicas (12), como a própria DRC, pode favorecer a instalação, progressão e severidade de doenças infecciosas, como a periodontite, por intensificar a resposta imuno-inflamatória do hospedeiro (10).

As interleucinas (IL) são mediadoras-chave do processo inflamatório de diversas doenças crônicas, como a DRC e a DP. A IL-1α e IL-1β apresentam atividades biológicas similares porque aumentam a adesão leucocitária às células endoteliais, permitem o acesso das células inflamatórias aos sítios de infecção, promovem a desgranulação dos neutrófilos, estimulam a

síntese de prostaglandinas pelos monócitos e fibroblastos, estimulam a liberação das metaloproteinases da matriz extracelular, que degradam componentes da matriz extracelular e promovem a reabsorção óssea (13, 14). Essas citocinas apresentam atividades pró-inflamatórias e catabólicas similares, pois se ligam a receptores comuns (15). Contudo, a IL-1*β* é cerca de 15 vezes mais potente que a IL-1*α* na mediação da reabsorção óssea *in vitro* (16, 17). A IL-1ra é o antagonista natural de ambas IL-1*α* e IL-1*β*, por se ligar aos receptores da IL-1, inibindo sua resposta biológica (18, 19).

Fatores de risco tradicionais de mortalidade em pacientes com DRC, tais como hipertensão, hipercolesterolemia e diabetes *mellitus*, não explicam completamente a alta taxa de mortalidade nesses pacientes (20-22). A concentração sérica de citocinas pró-inflamatórias, como IL-1, IL-6 e fator de necrose tumoral (TNF)-α está elevada nessa população e tem sido fortemente associada às complicações da DRC, aumentando seus níveis de mortalidade (23). Vários estudos mostram que os monócitos são cronicamente ativados em pacientes com DRC, dialisados ou não (24). Contudo, após a sessão de hemodiálise, os pacientes renais crônicos apresentam um aumento na produção de IL-1 pelos monócitos e altas concentrações plasmáticas de IL-1, principalmente IL-1*β* e IL-1ra (25). Dessa forma, a condição inflamatória parece constituir um significante fator de risco de mortalidade coadjuvante em pacientes com DRC. Nesse contexto, tem sido sugerido que a DP represente um indicador de mortalidade nesses pacientes, por aumentar os níveis de marcadores séricos de inflamação, como a proteína C reativa (10). Na DP, periodontopatógenos ativam a produção das interleucinas pró-inflamatórias, como a IL-1, que modula a degradação de componentes da matriz extracelular e a reabsorção do osso, que compõem os tecidos periodontais (26). Níveis aumentados de IL-1 foram encontrados na gengiva (27-29) e em sítios ativos de pacientes com periodontite crônica (30), e diminuídos no fluido gengival após tratamento da DP (13, 30, 31).

O *cluster* da *IL1* é constituído pelos genes *IL1A*, *IL1B* e *IL1RN*, localizados no braço longo do cromossomo 2, que codificam as citocinas IL-1α, IL-1*β* e IL-1ra (antagonista do receptor da IL-1), respectivamente. Os genes *IL1A* e *IL1B* apresentam polimorfismos de base única (*Single Nucleotide Polymorphisms* - SNPs). Polimorfismos são variações genéticas comumente encontradas na população que geram formas gênicas variantes (alelos), cuja freqüência do alelo mais raro é maior que 1% na população (32). Há polimorfismos que influenciam a atividade de fatores reguladores da resposta inflamatória, alterando sua expressão, podendo estar associados à suscetibilidade a doenças (33). Diferenças individuais nos níveis de interleucinas, relacionados aos diferentes graus de susceptibilidade a doenças inflamatórias,

podem ser atribuídas a alelos de genes polimórficos (34-36). O gene *IL1A* apresenta um polimorfismo na posição -889, e o *IL1B*, na posição -511 (C/T) e +3954 (C/T), a partir do sítio de início de transcrição. O polimorfismo *IL1B*+3954 foi reportado estar em forte desequilíbrio de ligação com o polimorfismo *IL1A*-889 em diversas populações (37). No íntron 2 do gene *IL1RN*, há um polimorfismo de variação do número de repetições (86 pb) em tandem (*Variable Number of Tandem Repeats* - VNTR). Na posição -889 do promotor do gene *IL1A* foi descrito um polimorfismo caracterizado pela substituição de uma base citosina (C) por uma timina (T), originando dois possíveis alelos: alelo C e alelo T (38). Estudos demonstraram que pacientes que carregam o alelo T podem produzir níveis mais elevados da proteína IL-1α (39). Na posição -511 do promotor e na posição +3954 do exon 5 do gene *IL1B* foram descritos polimorfismos bi-alélicos, caracterizados pela substituição de uma base C por T (40). Os alelos T dos dois polimorfismos foram relacionados à produção aumentada da proteína IL-1β (40). Foi observado que dois destes polimorfismos (alelos *IL1A*-889*T e *IL1B*+3954*T), quando juntos, estiveram associados à presença de periodontite severa em adultos não-fumantes (12). Posteriormente, foi demonstrado desequilíbrio de ligação entre esses dois *loci* polimórficos (41). Pacientes que portavam esse haplótipo, composto por alelos T de ambos os polimorfismos, foram denominados genótipo-positivo (12). A presença de um dos polimorfismos (*IL1*β +3954*T), que compõe o genótipo-positivo, está relacionada a uma produção duas a quatro vezes maior da IL-1β *in vitro* (12, 42). No intron 2 do gene *IL1RN,* o polimorfismo VNTR gera cinco possíveis alelos, que correspondem à presença de 2, 3, 4, 5 ou 6 repetições de 86 pb (43). O alelo *IL1RN**2 (intron 2) foi associado à produção diminuída da proteína IL-1ra (44, 45). Além disso, células mononucleares de portadores desse polimorfismo demonstraram produzir concentrações elevadas de IL-1β *in vitro* (46). É provável que este polimorfismo aumente o nível de IL-1β, resultando em um desequilíbrio na relação IL-1β/IL-1ra o que conduz a um aumento da suscetibilidade ou uma resposta mais intensa para as doenças inflamatórias. O alelo *IL1RN**2 pode ser um indicador de doença inflamatória; porém, não necessariamente estar ligado a sua causa (47). Esses polimorfismos são funcionais (37, 48), pois regulam os níveis de expressão das citocinas correspondentes, e vêm sendo associados a diversas doenças inflamatórias e degenerativas, como a o câncer gástrico (49-51), doença intestinal inflamatória (45), lúpus eritematoso sistêmico (52), síndrome de Sjögren's (53), doença arterial coronariana (54), doenças neurodegenerativas (55). Também, polimorfismos nos genes da IL-1 foram associados à DRC em estágio final (25, 56-58) e à DP crônica (59-67).

Pacientes com DRC são particularmente susceptíveis ao desenvolvimento de complicações, como a DP (9, 68-72). Por outro lado, a DP constitui um foco infeccioso que pode influenciar a progressão da DRC, por elicitar potencialmente a exacerbação da inflamação sistêmica (71). A DRC e a DP são doenças de natureza imuno-inflamatória, com eventos moleculares análogos; assim, podem apresentar fatores genéticos comuns de predisposição. Polimorfismos funcionais, que alteram a taxa de expressão de genes da resposta imuno-inflamatória do hospedeiro, como a IL-1, podem influenciar sua predisposição a doenças sistêmicas e locais, com mecanismos fisiopatogênicos similares.

OBJETIVOS

O objetivo deste estudo foi investigar a associação de polimorfismos funcionais em genes da IL-1, e a sua expressão, com a suscetibilidade à DRC e à DP.

Objetivo específico 1

Investigar a associação dos polimorfismos *IL1A* C-889T, *IL1B* C-511T, *IL1B* C+3954T e *IL1RN* (86 pb - intron 2) com a susceptibilidade à DRC e à DP.

Objetivo específico 2

Comparar os níveis de transcritos dos genes *IL1A*, *IL1B* e *IL1RN* entre os grupos com e sem DRC, com e sem DP, e de acordo com genótipos.

Association of *IL1* gene polymorphisms and transcript levels with periodontal disease and chronic kidney disease

A. P. Ribeiro Braosi[1]; C. M. de Souza[1]; S. M. Luczyszyn[1]; A. J. Dirschnabel[1]; A. D. Doetzer[1]; A. R. Ávila[2]; A. C. Dominguez[2]; M. C. Riella[3]; M. Olandoski[4]; C. M. Probst[2]; G. P. Garlet[5]; R. F. Pecoits-Filho[4]; P. C. Trevilatto[4]

[1] Graduate students of the Center for Health and Biological Sciences at Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, PR, Brazil.

[2] Researcher at Instituto de Biologia Molecular do Paraná (IBMP) - FIOCRUZ, Curitiba, PR, Brazil.

[3] Professor of the Center for Health and Biological Sciences at Pontifícia Universidade Católica do Paraná (PUCPR), and President of Renal, Diabetes and Hypertension Research Center of Pro-Renal Foundation (Pro-Renal), Curitiba, PR, Brazil.

[4]Professor of the Center for Health and Biological Sciences at Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, PR, Brazil.

[5]Department of Biological Sciences, School of Dentistry of Bauru, University of São Paulo, Bauru, São Paulo, Brazil; Bauru, SP, Brazil.

Running title: *IL1* gene polymorphisms and chronic kidney disease and periodontitis.

Key Words: chronic kidney disease; periodontal disease; interleukin-1, genetic polymorphisms, gene transcripts.

Corresponding author:
Paula Cristina Trevilatto, DDS, PhD
Center for Health and Biological Sciences (CCBS)
Pontifícia Universidade Católica do Paraná (PUCPR)
Rua Imaculada Conceição, 1155
80215-901
Curitiba, PR, Brazil
Phone/Fax: +55 (41) 3271-2618 / +55 (41) 3271-1657
e-mail: pctrev@yahoo.com.br

Abstract

Background and Objective: Chronic kidney disease (CKD) and periodontal disease (PD) are complex inflammatory disturbances, which may be influenced by environmental and genetic. factors. Interleukin (IL)-1 genes code for inflammatory mediators that play a central role in the physiopathogenesis of both diseases. Functional polymorphisms In *IL1* genes modulate their expression levels and have been associated with susceptibility to several immune-inflammatory conditions. The aim of this study was to investigate the association of the functional polymorphisms in the *IL1* genes and gene transcripts levels with susceptibility to CKD and PD.

Material and Methods: The study population consisted of 246 individuals, mean age 44.8 years, and was divided into: *group 1* (64 patients without CKD and without PD), *group 2* (58 patients without CKD and with PD), *group 3* (52 patients with CKD and without PD) and *group 4* (72 patients with CKD and with PD). Genomic DNA was obtained from cells of oral mucosa and the polymorphisms *IL1A*C-889T, *IL1B*C-511T, *IL1B*C+3954T and *IL1RN* (intron 2) were analyzed by PCR-RFLP. Interleukin-1 gene transcripts from gingival tissues were analyzed by real time-PCR technique. The risk associated with genotypes, alleles and haplotypes was calculated as the odds ratio (OR) with 95% confidence intervals (CI). To access possible differences in the intensity of mRNA expression among the groups was used ANOVA (p<0.05).

Results: The *IL1RN**1 allele was associated with a roughly 3-fold increased risk of CKD (OR 2.86 95% CI=1.1-7.4, p=0.045). The *IL1RN**2 allele was associated with PD in CKD patients (OR 3.53 95% CI=1.5-8.4, p=0.005), as well as *IL1B*+3954*T allele associated with CKD when patients with PD were analyzed (OR 1.96 95% CI=1.10-3.48, p=0.030). Increased levels of transcripts of *IL1A*, *IL1B* and *IL1RN* genes were observed in PD patients, although no statistically significant differences were observed between groups without and with CKD.

Conclusion: It was observed an association between *IL1* gene polymorphisms and susceptibility to CKD and PD. Moreover, higher levels of *IL1* gene transcripts were found in PD patients. The present study suggests that the association of *IL1* gene cluster polymorphisms with PD and CKD maybe remains in the fact that both are immune-inflammatory diseases which share common mechanisms influencing their onset, severity and progression.

Introduction

Chronic kidney disease (CKD) is an inflammatory progressive illness characterized by the gradual and usually permanent loss of kidney function over time. Kidney disease is divided into five stages of increasing severity (1, 2). Independently of its etiology, CKD can progress to an advanced stage, or renal disease stage 5, in which predominate signs and symptoms of uremia (uremic syndrome), designated End Stage Renal Disease (ESRD). Presence of ESRD indicates the necessity of a replacement therapy, such as dialysis or renal transplantation (1, 2). Many diseases can lead to CKD, fast or slowly, but always progressively. Chronic kidney disease can result from a wide spectrum of diseases such as diabetes, hypertension, glomerulonephritis, and autoimmune disorders, even though its clinical manifestations are largely independent on the initial insult that damaged the kidneys (3-5). Many of the CKD patients are misdiagnosed while others die before treatment. The number of individuals with kidney failure treated by dialysis and transplantation has increased dramatically in the United States, from 209,000 in 1991, exceeded 320,000 in 1998 to 472,000 in 2004, and is expected to surpass 650,000 by 2010 (6-9). The incidence of CKD stage 5 was reported to be greater than the prevalence of CKD stage 1, which was of 19.2 millions in the United States (9). This fact is probably due to a higher survival and acceptance to attend treatment programs (7). The number of new cases per year is 9.6% in USA, and about 8.8% in Brazil (9). In 2005, the prevalence of CKD patients in Brazil was about 2 millions (9). According to data from the Brazilian Society of Nephrology, in 2008, there were 79,000 ESRD patients receiving replacement therapy of renal function, being of these, 35,928 (89.4%) in hemodialysis (9).

Inflammation and infection seem to be important causes of morbidity and mortality in CKD patients (11), and they seem to be more prone to infection diseases, such as hepatitis (12) and periodontal disease (PD) (13, 14) than the general population. Patients in hemodialysis showed a higher prevalence of PD when compared to the general population (15, 15). Periodontal disease has been considered a CKD complication (1, 14) and its prevalence and severity are suggested to be increased in this population (14-21). Periodontitis can be a source of inflammation in hemodialysis patients (19). High levels of C-reactive protein, the major inflammation acute phase protein, were found either in patients with CKD (11, 15) as in individuals with PD (15, 21). Recently, our group has reported that patients with both diseases (CKD and PD) present significantly higher levels of C-reactive protein, which may impact the outcome of renal disease (18). Persistent systemic inflammation increases

cardiovascular morbidity/mortality in hemodialysis patients, which is the major cause of death in this population (22, 23). Thus, it was suggested that PD might be considered, more than a morbidity factor, a potential mortality indicator for those patients (22).

Periodontitis is an infectious chronic inflammatory disease that causes the destruction of periodontal supporting tissues and alveolar bone resorption. The disorder is initiated by periodontal pathogens which trigger the chronic inflammatory and immune responses that are thought to determine the clinical outcome of the disease (24). According to data of Brazilian Health Ministry (25), there are near 50% of 35 to 44 year-old Brazilian people with PD. Among Brazilian CKD patients, this number increases for 70 to 90% (26)

Chronic renal disease and PD are inflammatory conditions, in which polymorphonuclear and mononuclear cells synthesize a broad spectrum of proinflammatory cytokines (27, 28), such as interleukins (IL)-1α and β, and the IL-1 antagonist receptor (IL-1ra) (29). Interleukin-1α and IL-1β present similar biological activities, because they increase leukocyte adhesion to endothelial cells, enable the ingress of inflammatory cells into sites of infection, promote polymorphonuclear neutrophil degranulation, stimulate prostaglandin synthesis by monocytes and fibroblasts, stimulate the releasing of the matrix metalloproteinases which degrade proteins of the extracellular matrix, and promote bone resorption (30, 31). Although IL-1α and IL-1β are produced by different genes and present 27% of homology in the polypeptidic chain, they show similar catabolic and proinflammatory activities because they link to common receptors (32). However, IL-1β is about 15 times more potent than IL-1α in the mediation of bone resorption in vitro (33, 34). The IL-1ra is a natural antagonist of both IL-1α and IL-1β, acting by binding the IL-1 receptors, inhibiting biological response (35, 36). Several findings indicate that monocytes are chronically activated in CKD patients, in dialysis or not (37). Patients with CKD in hemodialysis sessions present an IL-1 increased production by monocytes and increased plasma concentrations of IL-1, mainly IL-1β and IL-1ra (28). In PD, elevated levels of IL-1 were also found in the gingiva and active chronic disease (33, 34, 38-40), and diminished in crevicular fluid after periodontal therapy (30, 41). However, sites surgically treated by flap debridement continued to show elevated levels of both IL-1α and β in gingiva after 6 months of periodontal treatment. The IL-1 gene cluster has been mapped to the long arm of chromosome 2 and consists of three genes: *IL1A*, *IL1B* and *IL1RN*, encoding IL-1α, IL-1β and IL-1ra proteins, respectively (42-43).

Contemporary, high-throughput sequencing efforts have identified a rich source of naturally occurring gene polymorphisms. Gene polymorphisms are a mechanism by which

individuals may exhibit variations in the sequence of genes (44). Most polymorphisms are single nucleotide exchanges (SNPs) that occur in a high frequency in the human genome (46, 47), which may affect the function of genes (47), and in turn may influence the individual susceptibility to diseases (43, 49). The *IL1A* gene presents a polymorphism in the position -889 (C/T); the *IL1B* gene, in the positions -511 (C/T) and +3954 (C/T), and the *IL1RN* gene in intron 2, where there is an 86-bp-variable-number-of-tandem-repeat (VNTR) polymorphism (43). These polymorphisms are considered functional (43) because they regulate the levels of the corresponding cytokines, and have been associated to several diseases, such as gastric cancer (50-52), bowel inflammatory disease (53), systemic lupus erythematosus (54), Sjögren's syndrome (55), coronary arterial disease (56), neurodegenerative diseases (57), and periodontitis (58-62).

Polymorphisms in the *IL1* gene cluster are functional candidates for the investigation of association with CKD and PD susceptibility. Though, the aim of this study was to investigate the association of polymorphisms *IL1A* (-889), *IL1B* (-511), *IL1B* (+3954), and *IL1RN* (intron 2) with renal and periodontal diseases. Also, the levels of those gene transcripts were measured by quantitative polymerase chain reaction (real-time PCR).

Material and Methods

Study population

A convenient sample of 246 unrelated, both sex, mean age 44.8 years (range 20 to 77) was selected from the Dental Clinics of Pontifical Catholic University of Paraná (PUCPR) and from the Dental Clinics of Pro-Renal Foundation. The patients were from the Southern region of Brazil (Table 1). Subjects completed personal, medical and dental history questionnaires, and within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study (approved by the Ethical Committee in Research at PUCPR, protocol 264/10184).

The sample was divided into four groups:

Group 1: 64 individuals without CKD [glomerular filtration rate > 90 mL/min, estimated according to the Modification of Diet in Renal Disease (MDRD) formula] (63), and without PD;

Group 2: 58 patients without CKD and with PD [clinical attachment level (CAL) $\geq$ 5 mm, in at least three teeth, in at least two quadrants] (64);

Group 3: 52 patients with CKD stage 5, in hemodialysis, and without PD;

Group 4: 72 patients with CKD stage 5, in hemodialysis, and with PD.

Subjects could not have any of the following exclusion criteria: chronic usage of anti-inflammatory drugs; HIV infection; immunosuppressive chemotherapy; history of any diseases known to severely compromise immune function (for groups 1 and 2); systemic active infection; current pregnancy or lactation; diseases of the oral hard or soft tissues, except caries (and periodontal disease for groups 1 and 3); use of orthodontic appliances, and present necrotizing ulcerative gingivitis and periodontitis.

Serum markers in CKD patients

Serum markers were measured according to the routine of dialysis clinics for CKD patients.

Clinical parameters of PD

Diagnosis of chronic PD was made on the basis of clinical parameters, such as probing pocket depth (PPD), and assessment of CAL. Measurements PPD and CAL were recorded at four points around each tooth. The following parameters were recorded: the gingival (GI) (65); the plaque (PI) (66), and the calculus index (CI) (67), and mobility (absent or present). The periodontal status of all subjects is shown in table 2.

DNA collection and purification

Cells were obtained through a mouthwash with 3% glucose solution and scraping of oral mucosa with a sterile spatula (68). DNA was extracted from epithelial buccal cells with ammonium acetate 10 M and EDTA 1 mM (69, 70).

Analysis of the IL1 gene cluster polymorphisms (RFLP-PCR/ VNTR)

Conditions of reaction and cycling were as follows: 1 µL of genomic DNA were used for PCR amplification in a reaction mixture containing 22.5 µL of PCR Supermix (Invitrogen Life Technologies, Carlsbad, CA, USA), and 1 µL of each primer (25 µM). All the reactions were performed in a *Techne* T-512 thermal cycler.

IL1A (C-889T) polymorphism

The oligonucleotides 5'-AAG CTT GTT CTA CCA CCT GAA CTA GGC-3' and 5'-TTA CAT ATG AGC CTT CCA TG-3' were used as primers, and the reaction was incubated for 5 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 51°C, and 1 min at 72°C, and a final extension at 72°C for 7 min. Restriction Fragment Length Polymorphism (RFLP) technique was performed in a final reaction volume of 20 µL with 10 µL aliquot of PCR products, and 3 U of N*co*I (Invitrogen Life Technologies, Carlsbad, CA, USA), digested at 37°C overnight (ON), to detect allele C (16 bp + 83 bp) and allele T (99 bp).

IL1B (C-511T) polymorphism

The oligonucleotides 5'-TGG CAT TGA TCT GGT TCA TC-3' and 5'-GTT TAG GAA TCT TCC CAC TT- 3' were used as primers. The cycles consisted of a initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 1 min, annealing at 51°C for 1 min, and elongation at 72°C for 1 min, with a final extension at 72°C for 7 min. RFLP technique was performed in a final reaction volume of 20 µL, with 10 µL aliquot of PCR products digested with 3 U of A*va*I at 37°C ON to detect allele C (114 bp + 190 bp) and allele T (304 bp).

IL1B (C+3954T) polymorphism - 5th exon

The oligonucleotides 5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3' and 5'-GCT TTT TTG CTG TGA GTC CCG-3' were used as primers. The cycles consisted of a initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 1 min, annealing at 59°C for 1 min, and elongation at 72°C for 1 min, with a final extension at 72°C for 7 min. RFLP technique was performed in a final reaction volume of 20 *µ*L, using 2 U of T*aq*I (Invitrogen Life Technologies, Carlsbad, CA, USA), at 65°C ON to detect allele C (97 bp + 85 bp) and allele T (182 bp). In both cases, a constant band of 12 bp was produced.

Restriction products were visualized by electrophoresis on vertical 10% non-denaturing polyacrylamide gels in 1X TBE (Tris-Borate, 89 mM boric acid, 2 mM EDTA), followed by silver staining. The genotypes were determined by comparing the restriction length polymorphism band patterns with a 1 kb plus DNA ladder (Invitrogen Life Technologies, Carlsbad, CA, USA).

IL1RN (VNTR) polymorphism - intron 2

The oligonucleotides 5'-CTC AGC CAA CAC TCC TAT-3' and 5'-TCC TGG TCT GCA GGT AA-3' were used as primers. The reaction was incubated for 5 min at 95°C, followed by 35 cycles of

1 min at 95°C, 1 min at 55°C and 1 min at 72°C, and additional time of 7 min at 72°C. In the second intron of *IL1RN* gene, five alleles are defined by different numbers of an 86-bp segment repeat. Genotypes were determined by comparing the size of the bands with the molecular weight ladder (1 kb plus DNA ladder, Invitrogen Life Technologies, Carlsbad, CA, USA), with separation into allele 1 (4 repeats - 412 bp), allele 2 (2 repeats - 240 bp), allele 3 (3 repeats - 326 bp), allele 4 (5 repeats - 498 bp), and allele 5 (6 repeats - 584 bp). PCR products were visualized by electrophoresis on vertical 10% non-denaturing polyacrylamide gels in 1X TBE (Tris-Borate, 89 mM boric acid, 2 mM EDTA), followed by silver staining. The genotypes were determined by comparing the band patterns with a 1 kb plus DNA ladder (Invitrogen Life Technologies, Carlsbad, CA, USA).

Analysis of cytokines mRNA expression

The pattern of mRNA expression for *IL1A*, *IL1B* and *IL1RN* was investigated by real-time PCR, and further associated with genotypes in the whole population, and within each group. Though, 15 patients from each group (n=60) were randomly scheduled for biopsies of gingiva (comprising crevicular and junctional epithelium and connective tissue). The gingival sites, from which biopsies were obtained, represented extreme phenotypes (groups 1 and 3: healthy sites, with no signs of inflammation and attachment loss; groups 2 and 4, active sites of disease, presenting bleeding and suppuration, and clinical attachment loss of at least 5 mm).

RNA extraction – cDNA synthesis

Total RNA from the gingival tissue biopsies was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the directions supplied by the manufacturer. Trizol (1 mL/mg tissue) was added to the biopsy material, vigorously shaken and macerated, incubated at room temperature for 5 minutes, and stored at -80°C. After defrosting, 0.2 mL of chlorophorm was added to each mililiter of the suspension, according to a previous report (71). The concentration of RNA was determined by the optical density at a wavelength of 260 nm using the GeneQuant (Pharmacia Amersham Biosciences, Piscataway, NJ, USA). Complementary DNA (cDNA) was synthesized using 1 µg/µL of RNA through a reverse transcriptase reaction, with the kit ImProm-II™ Reverse Transcriptase System (Promega Co., Wi, USA).

Real-Time PCR

Real-time-PCR quantitative mRNA analyses were performed in an ABI Prism 5700 Sequence Detection System using the SYBR-green fluorescence quantification system (Applied Biosystems, Warrington, UK) for quantitation of amplicons. The standard PCR conditions were 95°C (10 min), and then 40 cycles of 94°C (1 min), 56°C (1 min) and 72°C (2 min), followed by the standard denaturation curve. The sequences of human primers were designed using the PrimerExpress software (Applied Biosystems) using nucleotide sequences present in the GenBank database. PCR conditions for each target were conscientiously optimized with regard to primer concentration, absence of primer dimer formation, and efficiency of amplification of target genes and housekeeping gene control. SYBR Green PCR Master Mix (Applied Biosystems), 400 nM specific primers, and 2.5 ng of cDNA were used in each reaction. Threshold for positivity of real-time PCR was determined based in negative controls. Calculations for determining the relative level of gene expression were made according to the instructions from User's Bulletin (P/N 4303859) from Applied Biosystems, by reference to the *β*-actin in the sample, using the cycle threshold (Ct) method. Briefly, Ct is the point at which the exponential increase in signal (fluorescence) crosses a somewhat arbitrary signal level (usually 10 times background). The mean Ct values from duplicate measurements were used to calculate expression of the target gene, with normalization to an internal control (*β*-actin), and then compared with the target-internal control in control subjects to calculate fold increase expression, using the 2-ΔCt formula, also according to User's Bulletin. Negative controls without RNA and without reverse transcriptase were also performed. Results show one experiment representative of three. The levels of transcripts for *IL1A*, *IL1B*, and *IL1RN* genes according to the groups are shown in figure 1 (71).

Statistical Analysis

The significance of the differences in observed frequencies of each polymorphism between the groups was assessed by standard Chi-square (χ^2). Comparisons between two groups for nominal variables in tables 2x2 were performed using Fisher's exact test. The physical proximity of the polymorphisms justifies the simultaneous analysis as haplotypes. Statistical analysis was performed using statistical software BioEstat 2.0 for Windows, SPSS (Statistical Package for the Social Sciences) 10.0 for Windows (SPSS Inc, Chicago, IL), and the package ARLEQUIN 3.0 to calculate haplotype frequencies, gene heterozygosis, Hardy-Weinberg expectations and linkage disequilibrium. The risk associated with genotypes, alleles

and haplotypes was calculated as the odds ratio (OR) with 95% confidence intervals (CI). For continuous variables, T-student test was used to compare means for two groups. For non-parametric variables U Mann-Whitney test was used to assess differences between groups. Continuous variables were expressed as mean and standard deviation and their comparisons were performed using one-way analysis of variance (ANOVA). Normality condition of the variables in each group was evaluated using Shapiro-Wilks test.

The data regarding the positivity of expression of the investigated target gene between control groups and patients, was analyzed by the Fisher exact test. To access possible differences in the intensity of mRNA expression between control subjects and patients from the clinical groups, ANOVA was done. The differences in the intensity of fold increase mRNA in relation to the control group, and normalized by the housekeeping gene expression, between the patients from the clinical groups were analyzed by the U Mann–Whitney test. All statistical tests were performed with the GraphPad InStat 3.05 and GraphPad Prism 3.0 software (GraphPad Software Inc.).

For all the tests used, values of $p<0.05$ were considered statistically significant.

Results

Genotyping and haplotyping analysis

There was no significant difference (*NS*) in the genotype distribution between the groups for the polymorphisms *IL1A*-889 ($p=0.588$), *IL1B*-511 ($p=0.956$), *IL1B*+3954 ($p=0.081$), and *IL1RN* (intron 2) ($p=0.186$), neither was observed statistical variation in the allele frequency for the follow polymorphisms studied: *IL1A*-889 ($p=0.915$), *IL1B*-511 ($p=0.744$), *IL1B*+3954 ($p=0.117$). The genotype/allele distribution is shown in table 3.

When group 1 (control) was examined versus (*vs*.) group 3 (patients with CKD) for *IL1RN* (intron 2) polymorphism, statistically significant difference (*SSD*) was observed for genotype 1/1 versus 1/2 + 2/2 (OR 2.86 95% CI=1.1-7.4, $p=0.045$). Also, *SSD* was found when group 1 was examined *vs*. group 3, for the allele 1 *vs*. allele 2 (OR 3.92 95% CI=1.6-9.4, $p=0.002$). When group 3 (patients with CKD) was compared to group 4 (patients with CKD and PD), *SSD* was observed between allele 2 *vs*. allele 1 (OR 3.53 95% CI=1.5-8.4, $p=0.005$) for *IL1RN* (intron 2) polymorphism. For polymorphism *IL1B*+3954, when group 2 was analyzed *vs*. group 4, allele T *vs*. C (OR 1.96 95% CI=1.10-3.48, $p=0.030$), *SSD* was observed. When haplotypes were

considered, haplotypes containing *IL1RN**1 allele *vs*. the other haplotypes [group 1 *vs*. group 3, OR 4.57 95% CI=1.2-16.9, *p*=0.029], and haplotypes containing *IL1RN**2 allele *vs*. the other haplotypes [group 3 *vs*. group 4, OR 3.92 95% CI=1.1-14.5, *p*=0.055].

Genotyping and CKD serum markers

Considering CKD patients (groups 3 and 4), higher levels of serum markers were associated with *IL1RN**1 allele: iron (*p*=0.050), transferrin (*p*=0.034), erythrocytes (*p*=0.025), and mean index of hemoglobin saturation (*p*=0.006). For polymorphism *IL1B* (C-511T), allele T was associated with lower levels of erythrocytes (*p*=0.017). Higher levels of leukocytes were associated with *IL1RN**2 allele (*p*=0.035) and *IL1A*-889*T allele (*p*=0.031). Also, increased levels of alkaline phosphatase (*p*=0.035) was associated with *IL1RN**2 allele, *IL1B*+3954*C allele was related with augmented levels of the parathyroid hormone (PTH) (*p*=0.009), and *IL1B*-511*T allele was weakly related with CaxP product (*p*=0.080) and phosphorus (*p*=0.067) concentrations. *IL1B*-511*T associated with normalized protein catabolic rate (PCRN) (*p*=0.002), creatinin (*p*=0.006), urea post dialysis (*p*=0.032), and seric urea (*p*=0.002).

Genotyping and PD clinical parameters

When all groups were taken together, an association of *IL1B*+3954*T with GI (*p*=0.001) and CI (*p*=0.081) was observed. Analyzing only CKD patients (groups 3 and 4), it was noticed an association of *IL1RN**2 allele with GI (*p*=0.037) and CAL (*p*=0.015), and an association of *IL1A*-889*T with higher means of PI (*p*=0.052).

Quantitative analysis of expression of mRNA cytokines

There was a significant augment in the expression of the *IL1A, IL1B*, and *IL1RN* genes in groups with PD (groups 2 and 4). Although the levels of expression were higher in the group 4 in comparison with group 2, *SSD* was not observed (Fig. 1).

In relation to *IL1A* (C-889T) genotype distribution in the whole sample, no *SSD* was observed, although the levels of gene transcripts were increased in the presence of allele T. The same was observed within group 4, meanwhile in group 2 the expression decreased. For polymorphism *IL1B* (C-511T), genotype frequencies in the whole sample showed no *SSD*, although the levels of gene transcripts increased in genotypes presenting allele T. The same was observed within group 2. For polymorphism *IL1B* (C+3954T), genotype frequencies in the whole sample showed no *SSD*. In group 2, the levels of gene transcripts diminished in the

presence of allele T, although without *SSD*. For polymorphism *IL1RN* (intron 2), genotype frequencies in the whole sample showed no *SSD*. In group 2 the levels of gene transcripts were reduced while in group 4 they were increased in genotypes carrying allele 2, although without *SSD*.

Discussion

Chronic renal disease and periodontitis are multifactorial inflammatory diseases, which combine environmental and genetic factors influencing their progression and severity (14, 72-77).

Interleukin-1*α*, IL-1*β* and IL-1*ra* may function as immune modulators of inflammatory diseases (61). Although there are controversies regarding the genetic association studies, functional polymorphisms in the *IL1* gene cluster are told to contribute for alteration in the production of IL-1*α*, IL-1*β* and L-1*ra* proteins, so that variations in those genes, in a set with other factors, may have some consequences on disease progression and severity.

In spite of a high number of reports investigating the association of *IL1* gene cluster polymorphisms with susceptibility to CKD and PD (37, 78), this study is the first one to investigate the association of these cytokine gene polymorphisms with the susceptibility to PD in CKD patients.

In our results, *IL1RN**1 allele (the most frequent allele) was associated with a roughly 3-fold increased risk of CKD (group 1 *vs.* group 3). Similarly, studies have reported that the risk for ESRD aspects is higher in patients carrying the 1/1 genotype for the *IL1RN* (intron 2) polymorphism (79-81). On the other hand, differently from our results, renal patients which carry *IL1RN**2 were seen to present poor prognostic to CKD (74, 82-85). The *IL1RN**2 allele has been associated with aspects of cardiovascular diseases, such as single blood vessel disease, atherogenesis (56), atherosclerotic coronary disease (86), and acute coronary syndrome (87). The lower frequency of *IL1RN**2 allele observed for the study CKD patients may possibly be explained by the fact that allele 2 might predispose renal patients to death for cardiovascular reasons, before developing ESRD. Moreover, *IL1RN**2 allele was associated with PD in CKD patients (group 3 *vs.* group 4). These results replicate the previously reported association of *IL1RN**2 with chronic periodontitis (78, 88-90) in the general population. Regarding *IL1B*C+3954T polymorphism, when patients with PD were analyzed (group 2 *vs.* group 4), allele

T associated with CKD. Although some reports have identified none or a weak association with this polymorphism (85, 91), it is not surprising that allele T is associated with such an inflammatory disease, because it increases IL-1*β* expression (50), which is a potent pro-inflammatory cytokine mediator.

Renal patients in hemodialysis present an increased activation of proinflammatory cytokines, mainly IL-1 production, by the fact they are in a state of micro inflammation (92). Patients with CKD experience extremely high mortality, with cardiovascular causes accounting for about half of their death. In this context, non-traditional risk factors, such as chronic inflammation may represent an additional contribution to increased mortality of those patients (21, 76). Systemic inflammation could be still more exacerbated by the stimulation of peripheral blood mononuclear cells by periodontopathogens, which can lead to the synthesis and release of elevated plasma levels of proinflammatory cytokines, such as IL-1*α*, IL-1*β* and IL-1ra (93-95), and other serum markers. An association between some serum markers and specific IL-1 genotypes were observed in this study. Regarding *IL1RN* polymorphism, *IL1RN**1 allele was associated with higher levels of seric iron, transferrin, and erythrocytes, and the mean index of hemoglobin saturation levels. Pro-inflammatory cytokines, such as IL-1, may negatively influence iron utilization, thereby interfering with hemoglobin synthesis, decreasing transferrin saturation, which may lead to hypoferremia, that is the diminishing of iron seric levels (96, 97). Since the developing erythrocytes constitute the main iron-consuming tissue, the decrease in iron supply becomes a bottleneck for hemoglobin synthesis and eventually results in anemia (98). Reduced levels of the erythrocytes were also associated with *IL1B*-511*T allele, which augments IL-1*β* production (99), which has been reported to inhibit the maturation of erythrocytes (100). In addition, higher levels of leukocytes were associated with *IL1RN**2 and *IL1A-889**T alleles. The number of leukocytes has been related with IL-1 levels, independently of the IL-1 genotypes in CKD patients (101). Increased levels of alkaline phosphatase were associated with *IL1RN**2 allele, *IL1B+*3954*C allele was associated with higher seric levels of PTH, and *IL1B*-511*T allele was related with CaxP product and phosphorus concentrations, which may suggest a regulatory role for those polymorphisms in the active process of bone metabolism. Hemodialysis patients are chronically stimulated to produce IL-1*β* (102). It is possible that, independently on the genotype, renal patients produce increased levels of IL-1*β*, which represents a key regulator of PTH synthesis (103-104). The *IL1B*-511*T allele, which has been reported to augment the IL-1*β* (99), was associated to an increasing in the levels of PCRN and creatinin, which are nutritional markers, and of post-

dialysis and seric urea, adequacy of dialysis markers. No association between those markers and proinflammatory cytokines has been reported so far.

In regards of periodontal indexes, *IL1B*+3954*T was associated with higher means of GI and CI. In fact, *IL1B*+3954*T allele has been associated to higher destructive periodontal diseases, with more plaque accumulation, bleeding on probing and higher indexes of calculus (75, 105-111). The *IL1RN**2 allele was associated with increased values of GI and CAL in patients presenting CKD. Indeed, PD has been reported to be exacerbated in the presence of allele 2 in the general population (58, 61, 62, 88, 112). Moreover, *IL1A*-889*T was associated with higher means of PI in CKD patients. In fact, this allele has been associated with PI, PPD, CI, and a higher proportion of bleeding sites in a composite genotype with *IL1B*+3954*T (109, 113-115). IL-1 cytokines have been shown to play a central role in PD immune-inflammatory response (34, 75, 116).

When real-time PCR was used to evaluate the expression of IL-1 genes, increased levels of transcripts of *IL1A*, *IL1B* and *IL1RN* genes were observed in PD patients, although differences between groups 2 and 4 (without and with CKD) were *NS*. Also, some associations according to specific genotypes could be observed, although *NS*. The *IL1A*-889*T was associated with higher levels of gene transcripts within group 4 and when all groups were considered together. Unexpectedly, in group 2 the expression was decreased. The presence of the *IL1A*-889*T was associated with an augment in the expression of the corresponding cytokine in the blood of renal patients (37), and may represent an additional and stable marker of inflammation in CKD (11, 118). The *IL1B*-511*T was associated with higher levels of gene transcripts in the PD group, which has already been reported (117, 118), and even for other diseases, such as multiple myeloma (119) and cervical cancer (120). Differently from what has been reported (50, 75), diminished levels of gene transcripts were observed in PD patients presenting *IL1B*+3954*T allele. The *IL1RN**2 allele, which was observed to be associated with PD in this study, was also seen to be related with reduced levels of gene transcripts in group 2. Indeed, it was demonstrated that the presence of *IL1RN**2 was associated with a reduced production of IL-1ra (53, 122). On the other hand, it was noticed an increase in *IL1RN* gene transcript levels in patients from group 4. In fact, *IL1RN**1 was seen to be associated to ESRD in the study population.

An intriguing question to be addressed that still remains to be clarified is whether a systemic disease predisposes to the progression of a second one, or if there are common

mechanisms of genetic background predisposing to both. Chronic kidney disease seems to interfere with periodontitis progression (14, 123); on the other hand, inflammatory mechanisms exacerbated in PD patients could, in turn, influence CKD outcome (124). In this context, it seems that the *IL1RN* polymorphism is a potential genetic risk factor for inflammatory diseases, such as CKD and PD, compounding a common predisposing background. Once disease is installed, one local illness may interfere with the progression of a systemic one, and vice-versa. For polymorphisms, whose alleles play a small contribution to the phenotype, the population of CKD patients seems to be a good model to the study of complex diseases as PD. This particularly susceptible population to develop complications, such as inflammatory conditions, could allow the identification of genetic risk factors, just because they are more easily detected in those patients. The higher levels of *IL1* gene transcripts observed in patients with PD (groups 2 and 4), either of pro- or anti-inflammatory mediators, indicate an active process of disease, in which those modulators are involved in a direct or compensatory mechanism.

Conclusion

In summary, the present study suggests that the association of *IL1* gene cluster polymorphisms with PD and CKD maybe remains in the fact that both are immune-inflammatory diseases which share common mechanisms influencing their onset, severity and progression. Higher levels of *IL1* gene transcripts found in PD patients reflect the increased expression of inflammatory mediators in diseased sites, involved in disease exacerbation or controlling. Clinical parameters traditionally used to determine the outcome of inflammatory complex diseases, such as CKD and PD, seem to be, at least in part, dependent on specific genotypes.

Acknowledgements

This study was supported by grants from the Araucária Support Foundation for Scientific and Technological Development of Paraná (grant 5856) and National Counsel of Technological and Scientific Development (CNPq, grant 475770/2004-8).

References

1. Marakoglu I, Gursoy UK, Demirer S, Sezer H. Periodontal status of chronic renal failure patients receiving hemodialysis. *Yonsei Med J* 2003;**44**(4):648-652.

2. National Kidney Foundation. Definition and classification of stages of chronic kidney disease. *Am J Kidney Dis* 2002;**39**:S46-S75.

3. De Rossi SS, Glick M. Dental considerations for the patient with renal disease receiving hemodialysis. *J Am Dent Assoc* 1996;**127**:211-9.

4. Proctor R, Kumar N, Stein A, Porter D, et al. Oral and dental aspects of chronic renal failure. *J Dent Res* 2005;**84**:199-208.

5. Levey AS, Andreoli SP, DuBose T, Provenzano R, Collins AJ. Chronic kidney disease: common, harmful, and treatable— World Kidney Day 2007 - *J Am Soc Nephrol* 2007;**18**:374–378.

6. Hsu CY, Vittinghoff E, Lin F, Shlipak MG. The incidence of end-stage renal disease is increasing faster than the prevalence of chronic renal insufficiency. *Ann Intern Med* 2004;**20**;141:95-101.

7. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* **2003**;41:1-12.

8. Coresh J, Selvin E, Stevens LA, et al. Prevalence of Chronic Kidney Disease in the United States. *JAMA* 2007;**298**:2038-2047.

9. Schoolwerth AC, Engelgau MM, Hostetter TH et al. Links Chronic kidney disease: a public health problem that needs a public health action plan. *Prev Chronic Dis* 2006;**3**:A57.

10. Sociedade Brasileira de Nefrologia, 2008. Available in: http://www.sbn.org.br/Censo/2008/censoSBN2008.pdf.

11. Friedrich B, Alexander D, Janessa A, Häring H-U, Lang F, Risler T. Acute effects of hemodialysis on cytokine transcription profiles: Evidence for C-reactive protein-dependency of mediator induction. *Kidney Int* 2006;**70**,2124–2130.

12. Fabrizi F, Bunnapradist S, Martin P. HBV infection in patients with end-stage renal disease. *Semin Liver Dis* 2004;**24**:63-70.

13.Craig RG et al. Importance of periodontal disease in the kidney patient. *Blood Purif* 2002;**20**:113-119.

14. Borawski J, Wilczynska-Borawska M, Stokowska W et al. The periodontal status of pre-dialysis chronic kidney disease and maintenance dialysis patients. *Nephrol Dial Transplant* 2007;**22**:457-64.

15. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008;**35**:277-90. Epub 2008 Feb 20.

16. Wyatt CM, Winston J. Renal disease in patients with HIV. *Curr Infect Dis Rep* 2006;**81**:76-81.

17. Al-Wahadni A, Al-Omari MA. Dental diseases in Jordanian population on renal dialysis. *Quint Int* 2003;**34**:343-347.

18. Naugle K, Darby ML, Bauman DB et al. The oral health status of individuals on renal dialysis. *Ann Periodontol* 1998;**3**:197-205.

19. Rahmati MA, Craig RG, Homel P et al. Serum Markers of Periodontal Disease Status and Inflammation in Hemodialysis Patients. *Am J Kidney Dis* 2002;**40**:983-989.

20. Bayraktar G, Kurtulus I, Kazancioglu R, et al. Evaluation of periodontal parameters in patients undergoing peritoneal dialysis or hemodialysis. *Oral Dis* 2008;**14**:185-9.

21. Machado de Souza C, Ribeiro Braosi AP, Luczyszyn SM, et al. Association between Vitamin D Receptor Gene Polymorphisms and Susceptibility to Chronic Kidney Disease and Periodontitis. *Blood Purif* 2007 ;**25**:411-419.

22. Kshirsagar AV, Moss KL, Elter JR et al. Periodontal disease is associated with renal insufficiency in the Atherosclerosis Risk In Communities (ARIC) study. *Am J Kidney Dis* 2005;**45**:650-657.

23. Gonçalves S, Pecoits-Filho R, Perreto S, et al. Associations between renal function, volume status and endotoxaemia in chronic kidney disease patients. *Nephrol Dial Transplant* 2006;**21**:2788-2794.

24. Garlet GP, Martins JR W, Fonseca BAL et al. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *J Clin Periodontol* 2004;**31**:671-679.

25. Ministério da Saúde, Brasil. Condições de saúde bucal da população brasileira 2002-2003 - Resultados principais. Brasília, DF, 2004. Available in: http://www.saude.pr.gov.br/saudebucal/index.html, at 21/08/2004.

26.Gonçalves EM, Karam LALL, Milfont TS, Araújo MD, Santana JML, Lima DLF. Prevalence of Periodontitis in Maintenance Hemodialysis Patients. *J Bras Nefrol* 2007;**29**:115-119.

27. Hart TC. Genetic considerations of risk in human periodontal disease. *Curr Opin Periodontol* 1994; 3-11.

28. Dinarello CA. Interleukin-1 and interleukin-1 receptor antagonist production during haemodialysis: which cytokine is a surrogate marker for dialysis-related complications? *Nephrol Dial Transplant* 1995;**10**:25-28.

29. Gemmell E, Seymour GJ. Modulation of immune responses to periodontal bacteria. *Curr Opin Periodontol* 1994; 28-38.

30. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1α and -1β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. *J Periodont Res* 1990;**25**:156–163.

31. Mackay CR. Chemokines: immunology's high impact factors. *Nat Immunol* 2001;**2**:95-101.

32. Okada H, Murakami S. Cytokine expression in periodontal health and disease. *Crit Ver Oral Biol Med* 1998;**9**:248-266.

33. Stashenko P, Jandinski JJ, Fujiyoshi P, Reynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. *J Periodontol* 1991;**62**:504–509.

34. Stashenko P, Fujiyoshi P, Obernesser MS, Prostak L, Haffajee AD, Socransky SS. Levels of interleukin 1 beta in tissue from sites of active periodontal disease. *J Clin Periodontol* 1991;**18**:548-54.

35. Lennard AC. Interleukin-1 receptor antagonist. *Crit Rev Immunol* 1995;**15**:77

36. Vijgen L, Van Gysel M, Rector A, Thoelen I, et al. Interleukin-1 receptor antagonist VNTR-polymorphism in inflammatory bowel disease. *Genes Immun* 2002;**3**:400–406.

37. Le Meur Y, Lorgeot V, Aldigier JC, Wijdenes J, Leroux-Robert C, Praloran V. Whole blood production of monocytic cytokines (IL-1beta, IL-6, TNF-alpha, sIL-6R, IL-1Ra) in haemodialysed patients. *Nephrol Dial Transplant* 1999;**14**:2420-2426.

38. Silva N, Dutzan N, Hernandez M et al. Characterization of progressive periodontal lesions in chronic periodontitis patients: levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *J Clin Periodontol* 2008;**35**:206-14.

39. Honig J, Rordorf-Adam C, Siegmund C, Wiedemann W, Erard F. Increased interleukin-beta (IL-1 beta) concentration in gingival tissue from periodontitis patients. *J Periodontal Res* 1989;**24**:362–367.

40. Goutoudia P, Dizab E, Arvanitidouc M. Effect of periodontal therapy on crevicular fluid interleukin-1b and interleukin-10 levels in chronic periodontitis. *J Dent* 2004;**32**:511–520.

41. Reinhardt RA, Masada MP, Kaldahl WB, DuBois LM, Kornman KS, Choi J, Kalkwarf L, Allison AC. Gingival fluid IL-1 and IL-6 levels in refractory periodontitis. *J Clin Periodontol* 1993;**20**:225–231.

42. Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB. The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. *Genomics* 1992;**13**:654-657

43. Nicklin MJH, Weith A, Duff GW. A Physical Map of the Region Encompassing the Human Interleukin-1, Interleukin-1β, and Interleukin-1 Receptor Antagonist Genes. *Genomics* 1994;**19**:382-384.

44. de Jongh CM, Khrenova L, Kezic S, Rustemeyer T, Verberk MM, John SM. Polymorphisms in the interleukin-1 gene influence the stratum corneum interleukin-1 alpha concentration in uninvolved skin of patients with chronic irritant contact dermatitis. *Contact Dermatitis* 2008;**58**:263-268.

45. Human Genome Project Information. Available In: http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml 2007.

46. Venter JC et al. The Sequence of the Human Genome. *Science* 2001;**291**:1304–1351.

47. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999;**1**:3–19.

48. Hu S, Song QB, Yao PF, Hu QL, Hu PJ, Zeng ZR, Pang RP. No relationship between IL-1B gene polymorphism and gastric acid secretion in younger healthy volunteers. World J *Gastroenterol* 2005; **11**, 6549-6553.

49. McCarroll SA & Altshuler DM. Copy-number variation and association studies of human disease. *Nat Genet* 2007;**39**:37-42.

50. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992;**22**:396–402.

51. Danis VA, Millington M, Hyland VJ & Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. *Clin Exp Immunol* 1995;**99**:303-310.

52. Ruzzo A, Graziano F, Pizzagalli F *et al*. Interleukin 1B gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms in Helicobacter pylori-negative gastric cancer of intestinal and diffuse histotype. *Ann Oncol* 2005;**16**:887–892.

53. Tountas NA, Casini-Raggi V, Yang H, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. *Gastroenterology* 1999;**117**:806-813.

54. Blakemore AI, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* 1994; **37**: 1380–1385.

55. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezic B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopath* 1998;**87**:309–313.

56. Francis SE, Camp NJ, Dewberry RM et al. Interleukin-1 receptor antagonist gene polymorphism and coronary heart disease. *Circulation* 1999;**99**:861–866.

57. Davidson Y, Gibbons L, Pritchard A, et al. Genetic associations between cathepsin D exon 2 C-->T polymorphism and Alzheimer's disease, and pathological correlations with genotype. J *Neurol Neurosurg Psychiatry* 2006;**77**:515-517.

58. Tai H, Endo M, Shimada Y, et al. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J Clin Periodontol* 2002;**29:**882-888.

59. Li QY, Zhao HS, Meng HX et al. Association analysis between interleukin-1 family polymorphisms and generalized aggressive periodontitis in a Chinese population. *J Periodontol* 2004;**75**:1627-1635.

60. Moreira PR, de Sa AR, Xavier GM et al. functional interleukin-1 beta gene polymorphism is associated with chronic periodontitis in a sample of Brazilian individuals. *J Periodontal Res* 2005;**40:**306-311.

61. Scapoli C, Tatakis DN, Mamolini E, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis: interleukin-1 gene cluster polymorphisms*. J Periodontol* 2005;**76:**49-56.

62. Havemose-Poulsen A, Sørensen LK, Bendtzen K, Holmstrup P. Polymorphisms within the IL-1 gene cluster: effects on cytokine profiles in peripheral blood and whole blood cell cultures of patients with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *J Periodontol* 2007;**78**:475-92.

63. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;**130**:461-470.

64. Armitage GC: 1999 International Workshop for a classification of periodontal diseases and conditions. *Ann Periodontol* 1999;**4**:1-108.

65. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;**21**:533-51.

66. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22**:121-35.

67. Greene JC, Vermillion JR: The simplified oral hygiene index. *J Am Dent Assoc* 1964;68:7-9.

68. Trevilatto PC, Line SR: Use of buccal epithelial cells for PCR amplification of large DNA fragments. *J Forensic Odontostomatol* 2000;**18**(1):6-9.

69. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells*. Braz Dent J* 2007;18:148-52.

70. Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, de Souza AP, Line SR. Polymorphisms in the IL-1a and IL-1b genes are not associated with susceptibility to chronic periodontitis in a Brazilian population. *Braz J Oral Sci* 2003; **2**: 348-352.

71. Garlet GP, Martins W Jr, Fonseca BA, Ferreira BR, Silva JS. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *J Clin Periodontol*. 2004;**31**:671-679.

72. Diehl SR, Wang Y, Brooks CN, Burmeister JA, Califano JV, Wang S, Schenkein HA Linkage disequilibrium of interleukin-1 genetic polymorphisms with early-onset periodontitis. *J Periodontol* 1999;**70**:418-430.

73. Greenstein G, Hart TC. Clinical utility of a genetic susceptibility test for severe chronic periodontitis: a critical evaluation. *J Am Dent Assoc*. 2002;**133**:452-459.

74. Wetmore JB, Hung AM, Lovett DH, Sen S, Quershy O, Johansen KL. Interleukin-1 gene cluster polymorphisms predict risk of ESRD. *Kidney Int* 2005;**68**:278-84.

75. Ferreira, Jr SB, Trombone APF, Repeke CE et al. An interleukin-1β (il-1β) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and additively modulate the levels of IL-1β in diseased periodontal Tissues. *Infect Immun,* 2008;**76**:3725–3734.

76. Kovesdy CP, Kalantar-Zadeh K. Novel targets and new potential: developments in the treatment of inflammation in chronic kidney disease. *Expert Opin Investig Drugs* 2008;**17**:451-467.

77. Perunicic-Pekovic G, Pljesa S, Rasic-Milutinovic Z, Stankovic S, Ilic M, Maletic R. Inflammatory cytokines and malnutrition as related to risk for cardiovascular disease in hemodialysis patients. *Can J Physiol Pharmacol* 2008;**86**:205-209.

78. Laine ML, Farré MA, García-González MA et al. Risk factors in adult periodontitis: polymorphism in the interleukin-1 gene family. *Ned Tijdschr Tandheelkd* 2002;**109**:303-306.

79. Chen WC, Wu HC, Chen HY, Wu MC, Hsu CD, Tsai FJ. Interleukin-1beta gene and receptor antagonist gene polymorphisms in patients with calcium oxalate stones. *Urol Res* 2001; **29**:321-324.

80. Manchanda PK, Kumar A, Bid HK, Mittal RD. Interleukin-1beta and receptor antagonist (IL-1Ra) gene polymorphisms and the prediction of the risk of end-stage renal disease. *Biomarkers* 2006; **11**:164-73.

81. Mittal RD, Bid HK, Kumar A, Bhandari M. Association of urokinase gene 3'-UTR polymorphism with calcium oxalate nephrolithiasis. *J Endourol* 2006;**20**:157-60.

82. Lee SH, Ihm CG, Sohn SD et al. Polymorphisms in interleukin-1 beta and Interleukin-1 receptor antagonist genes are associated with kidney failure in Korean patients with type 2 diabetes mellitus. *Am J Nephrol* 2004; **24**:410-4.

83. Maruyama Y, Nordfors L, Stenvinkel P, et al. Interleukin-1 gene cluster polymorphisms are associated with nutritional status and inflammation in patients with end-stage renal disease. *Blood Purif*. 2005; **23**:384-93.

84. Shu KH, Cheng CH, Wu MJ, Chen CH, Lee WC. Interleukin 1 receptor antagonist and tumor necrosis factor-alpha gene polymorphism in patients with end-stage renal failure. *Ren Fail* 2005; **27**(1):53-7.

85. Uchiyama K, Naito K, Tsuchida M, et al. Impact of a genetic polymorphism of the interleukin-1 receptor antagonist on technique survival in peritoneal dialysis patients. *Blood Purif* 2005; **23**:450-8.

86. Dewberry RM, Crossman DC, Francis SE. Interleukin-1 Receptor Antagonist (IL-1RN) Genotype Modulates the Replicative Capacity of Human Endothelial Cells. *Circ Res* 2003; **92**;1285-1287

87. Ray KK, Camp NJ, Bennett CE, Francis SE, Crossman DC. Genetic variation at the interleukin-1 locus is a determinant of changes in soluble endothelial factors in patients with acute coronary syndromes. *Clin Sci* 2002; **103**:303-10.

88. Meisel P, Siegemund A, Dombrowa S, Sawaf H, Fanghaenel J, Kocher T. Smoking and polymorphisms of the interleukin-1 gene cluster (IL-1alpha, IL-1beta, and IL-1RN) in patients with periodontal disease. *J Periodontol* 2002;**73**:27-32.

89. Berdeli A, Emingil G, Gürkan A, Atilla G, Köse T. Association of the IL-1RN2 allele with periodontal diseases. *Clin Biochem* 2006;**39**:357-62.

90. Dashash, M., Drucker, D. B., Hutchinson, I. V., Bazrafshani, M. R. & Blinkhorn, A. S. Interleukin-1 receptor antagonist gene polymorphism and gingivitis in children. *Oral Dis* 2007;**13**,308-313.

91. Mittal RD, Bid HK, Manchanda PK, Kapoor R. Association of interleukin-1beta gene and receptor antagonist polymorphisms with calcium oxalate urolithiasis. *J Endourol* 2007;**21**:1565-1570.

92. Raj DS, Dominic EA, Pai A, Osman F, Morgan M, Pickett G, Shah VO, Ferrando A, Moseley P Skeletal muscle, cytokines, and oxidative stress in end-stage renal disease. *Kidney Int* 2005;**68**:2338-2344.

93. Kimmel PL, Phillips TM, Phillips E, Bosch JP: The effect of renal replacement therapy of cellular cytokine production in patients with renal disease. *Kidney Int* 1990;**38**:129–135.

94. Dinarello C: Cytokines: Agents provocateurs in hemodialysis ? *Kidney Int* 1992;**41**:683–694.

95. Balakrishnan VS, Schmid CH, Jaber BL, Natov SN, King AJ, Pereira BJG. Interleukin-1 Receptor Antagonist Synthesis by Peripheral Blood Mononuclear Cells: A Novel Predictor of Morbidity among Hemodialysis Patients. *J Am Soc Nephrol* 2000;**11**:2114–2121.

96. Gordeuk VR, Prithviraj P, Dolinar T, Brittenham GM. Interleukin 1 administration in mice produces hypoferremia despite neutropenia. *J Clin Invest* 1988;**82**:1934-1938.

97. Jeong KH, Lee TW, Ihm CG, Lee SH, Moon JY. Polymorphisms in two genes, IL-1B and ACE, are associated with erythropoietin resistance in Korean patients on maintenance hemodialysis. *Exp Mol Med* 2008;**40**:161-166.

98. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;**113**:1271–1276.

99. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998;**47**:195-8.

100. Takemasa A, Yorioka N, Yamakido M. Stimulation of interleukin-1 beta production may be involved in unresponsiveness to erythropoietin therapy. *Int J Artif Organs* 1996;**19**:633-637.

101. Kimmel PL, Phillips TM, Simmens SJ et al. Immunologic function and survival in hemodialysis patients. *Kidney Int* 1998;**54**:236-244.

102. Naumovic RT, Djukanovic LD, Ramic ZD, Ostric VZ. Relationship between IL-1beta and TNFalpha production by PBMC and clinical features of hemodialysis patients. *Ren Fail* 2000;**22**:195-204.

103. Higuchi T, Yamamoto C, Kuno T, Mizuno M, Takahashi S, Kanmatsuse K. Increased production of interleukin-1beta and interleukin-1 receptor antagonist by peripheral blood mononuclear cells in undialyzed chronic renal failure. *Nephron* 1997;**76**:26-31.

104. Ichizuka K, Morimoto T, Suzuki M, Sasaki Y, Kurihara H, Saito H, Yanaihara T. The effect of cytokines on parathyroid hormone-related protein (PTH-rP) production in human amnion cells. *Endocr J* 1999;**46**:479-486.

105. 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.

106. Armitage GC, Wu Y, Wang HY, Sorrell J, di Giovine FS, Duff GW. Low prevalence of a periodontitis-associated interleukin-1 composite genotype in individuals of Chinese heritage. *J Periodontol* 2000;**71**:164-171.

107. Jepsen S, Eberhard J, Fricke D, Hedderich J, Siebert R, Açil Y. Interleukin-1 gene polymorphisms and experimental gingivitis. *J Clin Periodontol* 2003;**30**:102-6.

108. Quappe L, Jara L, Lopez NJ. Association of interleukin-1 polymorphisms with aggressive periodontitis*. J Periodontol* 2004;**75**:1509-1515.

109. Lopez NJ, Jara L, Valenzuela CY. Association of interleukin-1 polymorphisms with periodontal disease. *J Periodontol* 2005;**76**:234-243.

110. Agrawal AA, Kapley A, Yeltiwar RK, Purohit HJ. Assessment of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for chronic periodontitis in Maharashtrian ethnicity. *J Periodontol* 2006;**77**:1515-1521.

111. Deinzer R, Weik U, Kolb-Bachofen V, Herforth A. Comparison of experimental gingivitis with persistent gingivitis: differences in clinical parameters and cytokine concentrations. *J Periodont Res* 2007;**42**:318–324.

112. Cullinan MP, Westerman B, Hamlet SM, et al. A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. *J Clin Periodontol* 2001;**28**:1137-1144.

113. Havemose-Poulsen A, Sørensen LK, Stoltze K, Bendtzen K, Holmstrup P. Cytokine profiles in peripheral blood and whole blood cell cultures associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *J Periodontol* 2005;**76**:2276-2285.

114. Konradsson K, Claesson R, van Dijken JW. Dental biofilm, gingivitis and interleukin-1 adjacent to approximal sites of a bonded ceramic. *J Clin Periodontol* 2007;**34**:1062-7.

115. Moreira PR, Costa JE, Gomez RS, Gollob KJ, Dutra WO. The IL1A (-889) gene polymorphism is associated with chronic periodontal disease in a sample of Brazilian individuals. *J Periodontal Res* 2007;**42**:23-30.

116. Genco RJ. Host responses in periodontal diseases: current concepts. *J Periodontol* 1992;**63**:338-355.

117. Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls. *J Clin Periodontol* 2008;**35**:754-767.

118. Chen H, Wilkins LM, Aziz N, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* 2006; **15**:519-529.

119. Abazis-Stamboulieh D, Oikonomou P, Papadoulis N, Panayiotidis P, Vrakidou E,Tsezou A. Association of interleukin-1A, interleukin-1B and interleukin-1 receptor antagonist gene polymorphisms with multiple myeloma. *Leuk Lymphoma* 2007;**48**:2196-2203.

120. Kang S, Kim JW, Park NH, Song YS, Park SY, Kang SB, Lee HP. Interleukin-1 beta-511 polymorphism and risk of cervical cancer. J Korean Med Sci. 2007;**22**:110-113.

121. Malaponte G, Libra M, Bevelacqua Y, et al. Inflammatory status in patients with chronic renal failure: the role of PTX3 and pro-inflammatory cytokines. *Int J Mol Med* 2007;**20**:471-481.

122. Andus T, Daig R, Vogl D, et al. Imbalance of the interleukin 1 system in colonic mucosa--association with intestinal inflammation and interleukin 1 receptor antagonist genotype 2. *Gut* 1997;**41**:651-657.

123. Cengiz MI, Bal S, Gökçay S, Cengiz K. Does periodontal disease reflect atherosclerosis in continuous ambulatory peritoneal dialysis patients? J Periodontol 2007;**78**:1926-1934.

124. Shultis WA, Weil EJ, Looker HC, Curtis JM, Shlossman M, Genco RJ, Knowler WC, Nelson RG. Effect of periodontitis on Overt Nephropathy and End-Stage Renal Disease in Type 2 Diabetes. Diabetes Care 2007;**30**:306-311.

Table 1. Baseline characteristics of the study population.

	Group 1 (n=64)	Group 2 (n=58)	Group 3 (n=52)	Group 4 (n=72)	*p* value
Ethnic Group n (%)					
Caucasoid	50 (78.1)	44 (75.9)	38 (73.1)	50 (69.4)	
Mulatto	10 (15.6)	13 (22.4)	12 (23.1)	18 (25)	0.735 *
Afro-American	4 (6.3)	1 (1.7)	2 (3.8)	4 (5.6)	
Age years (range) †	38.1±9.3 (20-70)	41.2±9.3 (20-61)	45.2±12.5 (23-74)	53.4±12.2 (26-77)	0.001 **
Gender n (%)					
Female	47 (73.4)	38 (65.5)	18 (34.6)	24 (33.3)	0.001 *
Male	17 (26.6)	20 (34.5)	34 (65.4)	48 (66.7)	

†Mean±Standart Deviation; * Chi-square; **ANOVA.

The difference observed among groups in the mean age and gender is due to most CKD patients being older and male.

Group 1: healthy patients. **Group 2:** without CKD and with PD. **Group 3:** with CKD and without PD. **Group 4:** presenting CKD and PD.

Table 2. Periodontal status of the study population.

Periodontal Status	Group 1 (n=64	Group 2 (n=58)	Group 3 (n=52)	Group 4 (n=72)	*p* value
Gingival Index †	0.2 ± 0.4	1.6 ± 1.0	0.4 ± 0.6	0.8 ± 0.9	<0.001*
Plaque Index †	0.3 ± 0.4	1.2 ± 1.0	0.6 ± 0.9	1.1 ± 1.0	<0.001*
Calculus Index †	0.2 ± 0.2	0.9 ± 0.9	0.4 ± 0.8	0.9 ± 0.9	<0.001*
PPD [a] (mm) †	1.5 ± 0.04	2.0 ± 0.5	1.3 ± 0.6	1.7 ± 0.7	<0.001*
CAL [b] (mm) †	1.5 ± 0.05	2.4 ± 0.9	1.3 ± 0.7	2.4 ± 1.4	<0.001*
Mobility (absence/presence)	62/1	33/23	37/12	36/33	<0.001**

[a]Probing pocket depth; [b]Clinical attachment level; †Mean ± Standart Deviation; *ANOVA; **Chi-square.

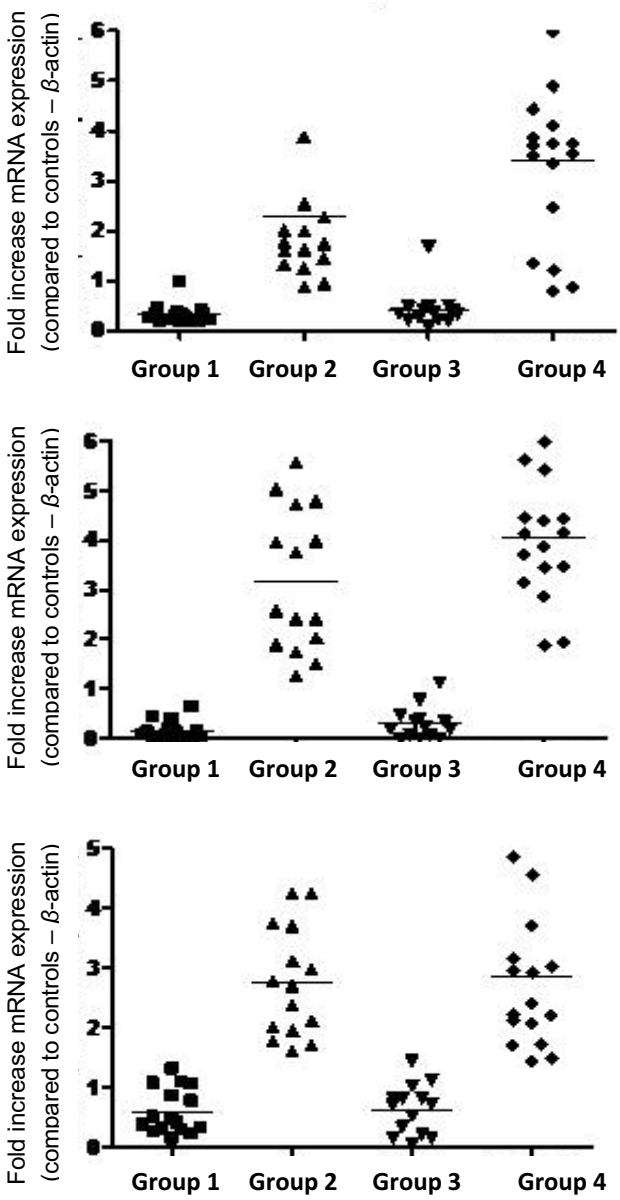
Group 1: healthy patients. **Group 2:** without CKD and with PD. **Group 3:** with CKD and without PD**. Group 4:** presenting CKD and PD.

Table 3. Allelic and genotypic distribution of *IL1* gene cluster polymorphisms.

Polymorphisms	Group 1 n=64 (%)	Group 2 n=58 (%)	Group 3 n=52 (%)	Group 4 n=72 (%)	Chi-square *p* value
***IL1A* (C-889T)**					
Genotypes					
T T	5 (7.8)	6 (10.3)	2 (3.8)	3 (4.2)	χ^2=4.7
T C	27 (42.2)	20 (34.5)	22 (42.4)	35 (48.6)	*p*=0.588
C C	32 (50.0)	32 (55.2)	28 (53.8)	34 (47.2)	
Alleles					
T	37 (28.9)	32 (27.6)	26 (25.0)	41 (28.5)	χ^2=0.51
C	91 (71.1)	84 (72.4)	78 (75.0)	103 (71.5)	*p*=0.916
***IL1B* (C-511T)**					
Genotypes					
T T	12 (18.8)	13 (22.4)	10 (19.2)	15 (20.8)	χ^2=1.5
T C	28 (43.7)	29 (50.0)	25 (48.1)	35 (48.6)	*p*=0.956
C C	24 (37.5)	16 (27.6)	17 (32.7)	22 (30.6)	
Alleles					
C	76 (59.4)	61 (52.6)	59 (56.7)	79 (54.9)	χ^2=1.2
T	52 (40.6)	55 (47.4)	45 (43.3)	65 (45.1)	*p*=0.744
IL1B (C+3954T)					
Genotypes					
T T	6 (9.4)	6 (10.3)	2 (3.9)	12 (16.7)	χ^2=11.2
T C	20 (31.3)	11 (19.0)	21 (40.4)	23 (31.9)	*p*=0.081
C C	38 (59.3)	41 (70.7)	29 (55.7)	37 (51.4)	
Alleles					
C	96 (75.0)	93 (80.2)	79 (76.0)	97 (67.4)	χ^2=5.9
T	32 (25.0)	23 (19.8)	25 (24.0)	47 (32.6)	*p*=0.117
IL1RN* (VNTR- INTRON 2*)					
Genotypes					
1 1	41 (64.0)	39(67.2)	45 (86.5)	48 (66.7)	χ^2=12.5
1 2	16 (25.0)	12 (20.7)	7 (13.5)	18 (25.0)	*p*=0.186
1 4	1 (1.6)	0 (00.0)	0 (00.0)	0 (00.0)	
Alleles					
1	99 (77.3)	90 (77.6)	97 (93.3)	114 (79.2)	χ^2=12.2
2	28 (21.9)	26 (22.4)	7 (6.7)	29 (20.1)	*p*=0.031
4	1 (0.8)	0 (00.0)	0 (00.0)	1 (0.7)	

Group 1: healthy patients. Group 2: without CKD and with PD. Group 3: with CKD and without PD. Group 4: presenting CKD and PD.

Figure 1. Quantitative expression of Interleukins (IL-1) in periodontal disease (PD) and chronic kidney disease (CKD) patients. Total RNA was extracted, and levels of IL-1 were measured quantitatively by real-time-PCR SYBR-Green System. The results are presented as the fold increase of expression of the individual mRNAs, with normalization to *β*-actin, when compared with the target–internal control of control subjects using the cycle threshold (Ct) method. Statistically significant difference was observed between groups without and with PD.



CONCLUSÃO

i) O alelo *IL1RN**1 foi associado a um risco cerca de 3 vezes maior para o desenvolvimento da DRC. Além disto, o alelo *IL1RN**2 foi associado à DP em pacientes com DRC, e o alelo *IL1B*+3954*T associou-se com a DRC em indivíduos com DP. O presente estudo sugere que a associação de polimorfismos no conjunto de genes da *IL1* com a DRC e a DP pode ser explicada por ambas as doenças apresentarem natureza imuno-inflamatória, compartilhando mecanismos fisiopatológicos comuns, que influenciam sua instalação, severidade e progressão.

ii) Níveis aumentados dos transcritos gênicos *IL1A*, *IL1B* e *IL1RN* foram observados em pacientes com DP; contudo independentemente de genótipos específicos. Altos níveis de transcritos gênicos da *IL1* encontrados em indivíduos com DP refletem a expressão aumentada dos mediadores inflamatórios em sítios com doença ativa, estando envolvidos tanto com a exacerbação quanto com o controle da doença.

iii) Parâmetros clínicos tradicionalmente usados para determinar o prognóstico de doenças inflamatórias complexas, tais como a DP e DRC, parecem ser, pelo menos em parte, dependentes de genótipos específicos.

REFERÊNCIAS

REFERÊNCIAS

1. Sobrado-Marinho JS, Tomás-Carmona I, Loureiro A, Limeres-Posse J, García-Caballero L, Diz-Dios P. Oral health status in patients with moderate-severe and terminal renal failure. *Med Oral Patol Oral Cir Bucal* 2007;**12**:E305-10.

2. Taal MW, Brenner BM. Kidney Int 2008;**73**:1216–1219.

3. Iino Y. Definition of CKD and classification of CKD stage. *Nippon Rinsho* 2008;**66**:1645-9.

4. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;**39**:S 1-266.

5. Vara-González L, Martín Rioboó E, Ureña Fernández T, Dalfó Baqué A, Flor Becerra I, López Fernández V. Prevalence of Chronic Kidney Disease in Hypertensive Patients Under Treatment at Primary Care Health Centres in Spain and the Monitoring of Their Blood Pressure. The DISEHTAE Study. *Aten Primaria* 2008;**40**:241-5.

6. Romão-Jr JE. Epidemiologia da doença renal crônica no Brasil – Cenário atual, 2004. Disponível em: http://www.sbn.org.br/Censo/epidemiologia2004.pps.

7. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J Clin Periodontol* 2008;**35**:277-90. Epub 2008 Feb 20.

8. Naugle K, Darby ML, Bauman DB et al. The oral health status of individuals on renal dialysis. *Ann Periodontol* 1998;**3**:197-205.

9. Craig RC, Spittle MA, Levin NW. Importance of Periodontal Disease in the Kidney Patient. *Blood Purification* 2002;**20**:113-119.

10. Machado de Souza C, Ribeiro Braosi AP, Luczyszyn SM, Rodrigues Ávila A, Barbosa de Brito Jr R, Aparecido Ignácio S, Macagnan Probst C, Riella MC, Santos Sotomaior V, Távora Mira M, Pecoits-Filho R, Trevilatto PC. Association between Vitamin D Receptor Gene Polymorphisms and Susceptibility to Chronic Kidney Disease and Periodontitis. *Blood Purif* 2007;**25**:411-419.

11. Garlet GP, Martins JR W, Fonseca BAL et al. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. *J Clin Periodontol* 2004;**31**:671-679.

12.Kornman KS *et al*. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;**24**:72-77.

13.Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1α and -1β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. *J Periodont Res* 1990;**25**:156–163.

14. Mackay CR. Chemokines: immunology's high impact factors. *Nat Immunol* 2001;**2**:95-101

15. Okada H, Murakami S. Cytokine expression in periodontal health and disease. *Crit Ver Oral Biol Med* 1998;**9**:248-266.

16. Stashenko P, Jandinski JJ, Fujiyoshi P, Reynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. *J Periodontol* 1991;**62**:504–509.

17. Stashenko P, Fujiyoshi P, Obernesser MS, Prostak L, Haffajee AD, Socransky SS. Levels of interleukin 1 beta in tissue from sites of active periodontal disease. *J Clin Periodontol* 1991;**18**:548-54.

18. Lennard AC. Interleukin-1 receptor antagonist. *Crit Rev Immunol* 1995;**15**:77

19. Vijgen L, Van Gysel M, Rector A, Thoelen I, et al. Interleukin-1 receptor antagonist VNTR-polymorphism in inflammatory bowel disease. *Genes Immun* 2002;**3**:400–406.

20. Kovesdy CP, Kalantar-Zadeh K. Novel targets and new potential: developments in the treatment of inflammation in chronic kidney disease. *Expert Opin Investig Drugs* 2008;**17**:451-67.

21. Stenvinkel P, Lindholm B, Heimburger O. Novel approaches in an integrated therapy of inflammatory-associated wasting in end-stage renal disease. *Semin Dial* 2004;**17**:505-15.

22. Pecoits-Filho R, Lindholm B, Stenvinkel P. The malnutrition, inflammation, and atherosclerosis (MIA) syndrome -- the heart of the matter. *Nephrol Dial Transplant* 2002;**17** Suppl 11:28-31.

23. Kimmel PL, Phillips TM, Simmens SJ, et al. Immunologic function and survival in hemodialysis patients. *Kidney Int* 1998;**54**:236-44.

24. Le Meur Y, Lorgeot V, Aldigier JC, Wijdenes J, Leroux-Robert C, Praloran V. Whole blood production of monocytic cytokines (IL-1beta, IL-6, TNF-alpha, sIL-6R, IL-1Ra) in haemodialysed patients. *Nephrol Dial Transplant* 1999;**14**:2420-2426

25. Dinarello CA. Interleukin-1 and interleukin-1 receptor antagonist production during haemodialysis: which cytokine is a surrogate marker for dialysis-related complications? *Nephrol Dial Transplant* 1995;**10**:25-28.

26. Genco RJ. Host responses in periodontal diseases: current concepts. *J Periodontol* 1992;**63**:338-355.

27. Silva N, Dutzan N, Hernandez M et al. Characterization of progressive periodontal lesions in chronic periodontitis patients: levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *J Clin Periodontol* 2008;**35**:206-14.

28. Honig J, Rordorf-Adam C, Siegmund C, Wiedemann W, Erard F. Increased interleukin-beta (IL-1 beta) concentration in gingival tissue from periodontitis patients. *J Periodontal Res* 1989;**24**:362–367.

29. Goutoudia P, Dizab E, Arvanitidouc M. Effect of periodontal therapy on crevicular fluid interleukin-1b and interleukin-10 levels in chronic periodontitis. *J Dent* 2004;**32**:511–520.

30. Ferreira, Jr SB, Trombone APF, Repeke CE et al. An interleukin-1β (il-1β) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and additively modulate the levels of IL-1β in diseased periodontal Tissues. *Infect Immun,* 2008;**76**:3725–3734.

31. Reinhardt RA, Masada MP, Kaldahl WB, DuBois LM, Kornman KS, Choi J, Kalkwarf L, Allison AC. Gingival fluid IL-1 and IL-6 levels in refractory periodontitis. *J Clin Periodontol* 1993;**20**:225–231.

32. Thompson & Thompson. Genetics in Medicine, Sixth Edition. Editora Guanabara Koogan S.A. Rio de Janeiro, RJ. 2002;76-82.

33. Venter JC et al. The Sequence of the Human Genome. *Science* 2001;**291**:1304–1351.

34. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999;**1**:3–19.

35. Hu S, Song QB, Yao PF, Hu QL, Hu PJ, Zeng ZR, Pang RP. No relationship between IL-1B gene polymorphism and gastric acid secretion in younger healthy volunteers. *World J Gastroenterol* 2005; **11**, 6549-6553.

36. McCarroll SA & Altshuler DM. Copy-number variation and association studies of human disease. *Nat Genet* 2007;**39**:37-42.

37. de Jongh CM, Khrenova L, Kezic S, Rustemeyer T, Verberk MM, John SM. Polymorphisms in the interleukin-1 gene influence the stratum corneum interleukin-1 alpha concentration in uninvolved skin of patients with chronic irritant contact dermatitis. *Contact Dermatitis* 2008;**58**:263-8.

38. McDowell, T.L., Symons, J.A., Ploski, R., Forre, O. & Duff, G.W. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. *Arthritis Rheum* 1995;**38**:221-228.

39. Shirodaria, S., Smith, J., McKay, I.J., Kennett, C.N. & Hughes, F.J. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. *J Dent Res* 2000;**79**:1864-1869.

40. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992;**22**:396–402.

41. Gore, E.A., Sanders, J.J., Pandey, J.P., Palesch, Y. & Galbraith, G.M. Interleukin-1beta+3953 allele 2: association with disease status in adult periodontitis. *J Clin Periodontol* 1998;**25**:781-785.

42. Kornman KS; di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann Periodontol* 1998;**3**:327-338.

43. Tarlow JK, Clay FE, Cork MJ, Blakemore AI, McDonagh AJ, Messenger AG, Duff GW. Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *J Invest Dermatol* 1994;**103**:387-390.

44. Andus T, Daig R, Vogl D, Aschenbrenner E, Lock G, Hollerbach S, Kollinger M, Scholmerich J, Gross V. Imbalance of the interleukin 1 system in colonic mucosa--association with intestinal inflammation and interleukin 1 receptor antagonist genotype 2. *Gut* 1997;**41**:651-657.

45. Tountas NA *et al*. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. *Gastroenterology* 1999;**117**:806-813.

46. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998;**47**:195-198.

47. Laine ML, Leonhardt A, Roos-Jansaker AM, Pena AS, van Winkelhoff AJ, Winkel EG, Renvert S. IL-1RN gene polymorphism is associated with peri-implantitis. *Clin Oral Implants Res* 2006;**17**:380-385.

48.Raj SM, Chakraborty R, Wang N, Govindaraju DR. Linkage disequilibria and haplotype structure of four SNPs of the interleukin 1 gene cluster in seven Asian Indian populations. *Hum Biol* 2006;**78**:109-19.

49. Machado JC *et al*. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. *Gastroenterology* 2001;**121**:823-829.

50. Danis VA, Millington M, Hyland VJ & Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (*IL-1Ra) gene poly*morphi**sm**. *Clin Exp Immunol* 1995;**99**:303-310.

51. Ruzzo A, Graziano F, Pizzagalli F et al. Interleukin 1B gene (IL-1B) and interleukin 1 receptor antagonist gene (IL-1RN) polymorphisms in Helicobacter pylori-negative *gastric cancer o*f inte**st**inal and diffuse histotype. *Ann Oncol* 2005;**16**:887–892.

52. Blakemore AI, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* 1994;**37**:1380–1385.

53. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezic B. IL-1 receptor antagonist (IL-1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. *Clin Immunol Immunopath* 1998;**87**:309–313.

54. Francis SE, Camp NJ, Dewberry RM et al. Interleukin-1 receptor antagonist gene polymorphism and coronary heart disease. *Circulation* 1999;**99**:861–866.

55. Davidson Y, Gibbons L, Pritchard A, et al. Genetic associations between cathepsin D exon 2 C-->T polymorphism and Alzheimer's disease, and pathological correlations with genotype. *J Neurol Neurosurg Psychiatry* 2006;**77**:515-517.

56. Chen WC, Wu HC, Chen HY, Wu MC, Hsu CD, Tsai FJ. Interleukin-1beta g*ene and recept*or antagonist gene polymorphisms in patients with calcium oxalate stones. *Urol Res* 2001; **29:**321-324.

57. Manchanda PK, Kumar A, Bid HK, Mittal RD. Interleukin-1beta and receptor antagonist (IL-*1Ra) gene polymorphism*s and the prediction of the risk of end-stage renal disease. *Biomarkers* 2006; **11**:164-73.

58. Mittal RD, Bid HK, Kumar A, Bhandari M. Association of urokinase gene 3'-UTR polymorphism with calcium oxalate nephrolithiasis. *J Endourol* 2006;**20**:157-60.

59. Tai H, Endo M, Shimada Y, et al. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *J Clin Periodontol* 2002;**29**:882-888.

60. Li QY, Zhao HS, Meng HX et al. Association analysis between interleukin-1 family polymorphisms and generalized aggressive periodontitis in a Chinese population. *J Periodontol* 2004;**75**:1627-1635.

61. Moreira PR, de Sa AR, Xavier GM et al. functional interleukin-1 beta gene polymorphism is associated with chronic periodontitis in a sample of Brazilian individuals. *J Periodontal Res* 2005;**40**:306-311.

62. Scapoli C, Tatakis DN, Mamolini E, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis: interleukin-1 gene cluster polymorphisms. *J Periodontol* 2005;**76**:49-56.

63. Havemose-Poulsen A, Sørensen LK, Bendtzen K, Holmstrup P. Polymorphisms within the IL-1 gene cluster: effects on cytokine profiles in peripheral blood and whole blood cell cultures of patients with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *J Periodontol* 2007;**78**:475-92.

64. Laine ML, Farré MA, García-González MA et al. Risk factors in adult periodontitis: polymorphism in the interleukin-1 gene family. *Ned Tijdschr Tandheelkd* 2002;**109**:303-306

65. Meisel P, Siegemund A, Dombrowa S, Sawaf H, Fanghaenel J, Kocher T. Smoking and polymorphisms of the interleukin-1 gene cluster (IL-1alpha, IL-1beta, and IL-1RN) in patients with periodontal disease. *J Periodontol* 2002;**73**:27-32.

66. Berdeli A, Emingil G, Gürkan A, Atilla G, Köse T. Association of the IL-1RN2 allele with periodontal diseases. *Clin Biochem* 2006;**39**:357-62.

67. Dashash, M., Drucker, D. B., Hutchinson, I. V., Bazrafshani, M. R. & Blinkhorn, A. S. Interleukin-1 receptor antagonist gene polymorphism and gingivitis in children. *Oral Dis* 2007;**13**,308-313.

68. Borawski J, Wilczynska-Borawska M, Stokowska W et *al. The periodontal status of pre-*dialysis chronic kidney disease and maintenance dialysis patients. *Nephrol Dial Transplant* 2007;**22**:457-64.

69. Wyatt CM, Winston J. Renal disease in patients with HIV. *Curr Infect Dis Rep* 2006;**81:**76-81.

70. Al-Wahadni A, Al-Omari MA. Dental diseases in Jordanian population on renal dialysis. *Quint Int* 2003;**34**:343-347.

71. Rahmati MA, Craig RG, Homel P et al. Serum Markers of Periodontal Disease Status and Inflammat*ion in Hemodial*ysis P**a**tients. *Am J Kidney Dis* 2002;**40**:983-989.

72. Bayraktar G, Kurtulus I, Kazancioglu R, et al. Evaluation of periodontal parameters in pati*ents undergoing* perit**on**eal dialysis or hemodialysis. *Oral Dis* 2008;**14**:185-9.

ANEXOS

Outros artigos relacionados à tese submetidos à publicação:

ARTIGO 1:

ASSOCIATION BETWEEN VITAMIN D RECEPTOR GENE POLYMORPHISMS AND SUSCEPTIBILITY TO CHRONIC KIDNEY DISEASE AND PERIODONTITIS

Cleber Machado de Souza Ana Paula Ribeiro Braosi Sônia Mara Luczyszyn Andréa Rodrigues Ávila Rui Barbosa de Brito Jr. Sérgio Aparecido Ignácio Christian Macagnan Probst Miguel Carlos Riella Vanessa Santos Sotomaior Marcelo Távora Mira Roberto Pecoits-Filho Paula Cristina Trevilatto

Blood Purif 2007;25:411–419 ***Impact factor (IF) 1.822***

Abstract

Background/Aims: Chronic kidney disease (CKD) and periodontitis (PD) are serious public-health concerns. Vitamin D is a fat-soluble steroid hormone that interacts with its nuclear receptor (VDR) to regulate a variety of biological processes, such as bone metabolism, immune response modulation and transcription of several genes involved in CKD and PD disease mechanisms. The aim of this work was to investigate the association between polymorphisms in the VDR gene and end-stage renal disease (ESRD) and PD. Methods: 222 subjects with and without ESRD (in hemodialysis) were divided into groups with and without PD. Polymorphisms T*aq* I and B*sm* I in the VDR gene were analyzed by PCR restriction fragment length polymorphism. The significance of differences in allele, genotype and haplotype frequencies between groups was assessed by the 2 test (*p* value ! 0.05) and odds ratio (OR). Results: Allele G was associated with protection against ESRD: groups without versus with ESRD (GG) X (GA+AA): OR = 2.5, 95% CI = 1.4–4.6, *p* = 0.00; (G X A): OR = 1.5, 95% CI = 1.0–2.3, p = 0.02; (TG + CG) X (TA + CA): OR = 1.5, 95% CI = 1.0–2.3, *p* = 0.02. No association was observed between the study polymorphisms and susceptibility to or protection against PD. ***Conclusion:*** Allele G of the VDR *Bsm* I polymorphism was associated with protection against ESRD.

ARTIGO 2:

ANALYSIS OF THE ASSOCIATION OF POLYMORPHISM IN THE OSTEOPROTEGERIN GENE WITH SUSCEPTIBILITY TO CHRONIC KIDNEY DISEASE AND PERIODONTITIS.

Baioni CS, de Souza CM, Ribeiro Braosi AP, Luczyszyn SM, Dias da Silva MA, Ignácio SA, Naval Machado MA, Benato Martins WD, Riella MC, Pecoits-Filho R, Trevilatto PC.

J Periodontal Res. 2008 Oct;43(5):578-84 Impact factor (IF) 2.146

Background and Objective: Chronic kidney disease (CKD) is a complex disorder, which results in several complications involving disturbance of mineral metabolism. Periodontal disease is an infectious disease that appears to be an important cause of systemic inflammation in CKD patients. Periodontal disease is characterized by clinical attachment loss (CAL) caused by alveolar bone resorption around teeth, which may lead to tooth loss. Osteoprotegerin (OPG) is a key regulator of osteoclastogenesis. Polymorphisms are the main source of genetic variation, and single nucleotide polymorphisms (SNPs) have been reported as major modulators of disease susceptibility. The aim of this study was to investigate the association of a polymorphism located at position -223 in the untranslated region of the OPG gene, previously known as -950, with susceptibility to CKD and periodontal disease. Material and Methods: A sample of 224 subjects without and with CKD (in hemodialysis) was divided into groups with and without periodontal disease. The OPG polymorphism was analyzed by polymerase chain reaction and restriction fragment length polymorphism. Results: No association was found between the studied OPG polymorphism and susceptibility to CKD or periodontal disease. Conclusion: It was concluded that polymorphism OPG-223 (C/T) was not associated with CKD and periodontal disease in a Brazilian population. Studies on other polymorphisms in this and other genes of the host response could help to clarify the involvement of bone metabolism mediators in the susceptibility to CKD and periodontal disease.

ARTIGO 3

ORAL HEALTH IN BRAZILIAN PATIENTS WITH CHRONIC RENAL DISEASE.

Souza CM, Braosi AP, Luczyszyn SM, Casagrande RW, Pecoits-Filho R, Riella MC, Ignácio SA, Trevilatto PC.

Rev Med Chil. 2008 Jun;136(6):741-6. Epub 2008 Aug 26. Impact factor (IF) 0.345

BACKGROUND: Poor oral health status may have an impact on the health status of patients with chronic renal failure. AIM: To describe the oral health status of a group of Brazilian patients with chronic renal failure. MATERIAL AND METHODS: Retrospective review of the medical records of patients with chronic renal failure, of whom 13 (4.5%) were in a predialysis stage, 158 (55%) were on hemodialysis, 23 (8.4%) were on peritoneal dialysis and 92 (32.1%) were transplanted. General oral health, presence of dental calculus, and halitosis were recorded. The number of decayed, missed and filled teeth was analyzed by means of DMF-T (Decayed, Missed and Filled Teeth) index. RESULTS: The sample was composed of 152 men (53%) and 134 women (47%), aged 42+/-13 years. Oral health status was considered defective in most patients (83%). Eighty-seven percent had dental calculus and 55% had halitosis. Transplant patients reported significantly less halitosis (40.2%) than the rest of the groups. The DMF-T for the whole population was 20.6 and had a positive correlation with age. CONCLUSIONS: This group of patients with chronic renal failure presented a poor oral health status. Dental treatment programs for these patients should be implemented to avoid the exposure to dental pathogens.