SONIA MARA LUCZYSZYN

ANÁLISE DA ASSOCIAÇÃO DE POLIMORFISMO E DOS NÍVEIS DE TRANSCRIÇÃO DO GENE DA METALOPROTEINASE-1 COM A DOENÇA PERIODONTAL E A DOENÇA RENAL CRÔNICA

CURITIBA

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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: Prof[a]. Dra. Paula Cristina Trevilatto

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DEDICATÓRIA

Flávio e Felipe, meus meninos, meus amores...

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(Cleber Machado de Souza)

Sumário

SUMÁRIO

Resumo

RESUMO

A doença periodontal (DP) crônica é uma doença infecciosa de natureza inflamatória. Bactérias ativam mecanismos inflamatórios que acabam por destruir a matriz extracelular (MEC) dos tecidos de suporte periodontal. A doença renal crônica (DRC) é uma doença inflamatória progressiva, caracterizada pela destruição das unidades funcionais dos rins (néfrons) e que pode ser conseqüência de várias enfermidades, como a hipertensão arterial e o diabetes *mellitus*. Pacientes renais apresentam maior prevalência de DP e sua progressão acentuada nesses pacientes pode ser considerada uma das complicações da DRC. Embora as bactérias sejam essenciais para a iniciação da DP, há fatores modificadores que não causam a doença, mas amplificam alguns mecanismos de progressão e severidade, como o tabagismo, o estresse psicossocial, certas doenças sistêmicas (como é o caso da DRC) e polimorfismos genéticos. Polimorfismos são variações genéticas freqüentemente encontradas na população. Há polimorfismos que influenciam a atividade de fatores reguladores da resposta inflamatória, promovendo destruição tecidual por ação das metaloproteinases da matriz (MMPs). A MMP-1 apresenta grande participação na remodelação dos componentes da MEC e um desequilíbrio entre sua síntese e degradação pode resultar na destruição tecidual observada nas doenças inflamatórias. Níveis mais altos de MMP-1 foram encontrados no fluido e no tecido gengival de pacientes com periodontite, e no sangue e em tecidos de pacientes com DRC. A expressão excessiva ou inapropriada das MMPs tem sido associada a complicações da DRC e à progressão da DP. O polimorfismo *MMP1*-1607 (1G/2G) é considerado funcional por alterar a taxa de expressão da MMP-1. Assim, o objetivo do presente trabalho foi investigar a associação entre o polimorfismo no promotor do gene da MMP-1 (*MMP1*-1607, 1G/2G) e a suscetibilidade à DP e à DRC, bem como comparar o nível de transcritos entre os grupos. Para tanto, 254 indivíduos foram divididos em quatro grupos: *Grupo 1*, indivíduos sem DP e sem DRC (n=67); *Grupo 2*, com DP e sem DRC (n=60); *Grupo 3*, sem DP e com DRC em hemodiálise (n=52), e *Grupo 4*, com DP e com DRC em hemodiálise (n=75). O polimorfismo *MMP1*-1607 foi analisado pelas técnicas da reação em cadeia da polimerase (PCR) e comprimento de fragmento de restrição (RFLP). A análise dos transcritos gênicos de tecidos gengivais foi realizada por meio de PCR em tempo real. Observou-se ausência de associação do polimorfismo *MMP1*-1607 com a DP e com a DRC. Na análise da expressão gênica, houve aumento da expressão dos transcritos do gene *MMP1* nos grupos com DP. Esta expressão foi ainda maior em pacientes com DRC, embora esta diferença não tenha sido estatisticamente significativa. Dentro dos grupos, não houve diferença nos níveis de transcritos de acordo com os genótipos; embora na presença do alelo 2G, tenha sido observado aumento progressivo da expressão dos transcritos de *MMP1*. Concluiu-se que o polimorfismo *MMP1*-1607 não esteve associado à DP ou à DRC. Porém, os níveis de transcritos do gene *MMP1* mostraram-se aumentados nos grupos com DP.

Abstract

ABSTRACT

Chronic periodontal disease (PD) is an infectious illness of the oral cavity, characterized by inflammatory cell accumulation in the periodontal tissues. The bacteria activate inflammatory mechanisms promoting extracellular matrix (ECM) destruction of the supportive periodontal tissues. Chronic kidney disease (CKD) is an inflammatory progressive disorder characterized by the destruction of the kidneys' functional units (nephrons), which can result from a wide spectrum of diseases such as hypertension and diabetes. Renal patients show higher prevalence of PD and its pronounced progression in these patients can be considered a complication of CKD. Bacteria are essential to PD initiation; however, there are other factors that may not cause the disease, but amplify some progression and severity mechanisms such as tabagism, psychosocial stress, systemic diseases (*e.g.* CKD) and genetic polymorphisms. Polymorphisms are genetic variations frequently found in the population. There are polymorphisms that can influence the activity of regulator factors of inflammatory response, promoting tissue destruction by matrix metalloproteinases (MMPs) action. MMP-1 participates on EMC turnover, and a desequilibrium between its synthesis and degradation may result in tissue destruction, as observed in inflammatory diseases. Higher levels of MMP-1 were found in both fluid and gingival tissues from PD patients, and in both blood and tissues from CKD patients. Higher or inappropriate MMP expression has been associated with CKD complications and PD progression. *MMP1*-1607 (1G/2G) is considered a functional polymorphism as it can alterate MMP-1 expression rate. Thus, the aim of this study was investigate the association of *MMP1*-1607 (1G/2G) polymorphism and susceptibility to PD and CKD. The study population consisted of 254 individuals divided into 4 groups: *Group 1*, individuals without PD and without CKD (n=67); *Group 2*, with PD and without CKD (n=60); *Group 3*, without PD and with CKD under hemodialysis (n=52), and *Group 4*, with PD and with CKD under hemodialysis (n=75). Polymorphism *MMP1*-1607 was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). *MMP1* gene transcripts from gingival tissues were analyzed by real time-PCR technique. It was not observed evidence for association between *MMP1*-1607 polymorphism and PD or CKD. In the expression evaluation, increased levels of transcripts of *MMP1* gene were observed in PD patients gingival tissues. This expression was higher in CKD patients, but the difference was not statistically significant. Within the groups, it was not observed differences in the transcript levels according to genotypes; althought in the presence of 2G allele a progressive increase of *MMP1* expression was noted. It was concluded that *MMP1*-1607 polymorphism was not associated with PD or CKD. However, higher levels of *MMP1* gene transcripts were found in gingival sites with PD.

Introdução

INTRODUÇÃO

Metaloproteinases da Matriz

As metaloproteinases da matriz (MMPs) representam uma família de endopeptidades dependentes de cátions divalentes (Ca^{++}, Zn^{++}), capazes de degradar todos os componentes da matriz extracelular (MEC) e componentes da membrana basal (MB). Os componentes da MEC incluem vários tipos de colágeno, agrecan e elastina, e os da MB, a fibronectina e a laminina (Birkedal-Hansen, 1993; Nagase & Woessner, 1999). As MMPs são classificadas de acordo com a especificidade do substrato em colagenases, gelatinases, estromelisinas e *membrane-type* MMPs (Birkedal-Hansen, 1993; Birkedal-Hansen, 1995). A expressão gênica das MMPs é regulada por citocinas, como a interleucina-6 (IL-6), IL-1*β* e o fator de necrose tumoral-*α* (TNF-*α*) (Borden & Heller, 1997; Kossakowska *et al*., 1999). Mediadores inflamatórios do tipo *T-helper* 1 (Th1), como IL-1*β*, TNF-*α* e interferon (IFN)-*γ*, bem como a prostaglandina E2 (PGE2), têm sido descritos como reguladores positivos da expressão das MMPs (Harris *et al*., 2002; Katagiri & Takahashi, 2002). O efeito reverso é provavelmente exercido por citocinas Th2, tais como IL-4, IL-10 e IL-13 (Harris *et al*., 2002; Katagiri & Takahashi, 2002). Algumas MMPs podem ser consideradas como enzimas anti-fibróticas porque clivam colágeno, bem como pró-fibróticas porque ativam o fator transformador de crescimento-*β* (TGF-*β*) e a endotelina-1. Elas também podem favorecer a interação ectomesenquimal através da degradação do colágeno tipo IV (Ronco *et al*., 2007). A ativação das MMPs é regulada por um grupo de proteínas endógenas denominadas inibidores teciduais específicos de metaloproteinases (TIMPs), os quais são capazes de inibir, através de mecanismos não-específicos, quase todos os tipos de MMPs (Baker *et al.*, 2002). As MMPs desempenham um importante papel em eventos fisiológicos e patológicos durante o desenvolvimento embrionário e também no reparo tecidual do adulto (Sorsa *et al*., 1995; Uitto *et al*., 2003). Foi observada uma sobre-expressão de MMPs em várias doenças, como no câncer ovariano (Adley *et al*., 2008), na aterosclerose (Romero *et al*., 2008) e na osteoartrite (Hulejová *et al*., 2007). Por sua habilidade de degradar componentes da MEC, as MMPs participam da progressão de diversas doenças inflamatórias e degenerativas (Ronco *et al*., 2007).

Doença Periodontal

A doença periodontal (DP) é uma doença multifatorial, na qual a iniciação e progressão são dependentes de múltiplos fatores, tanto ambientais quanto genéticos (Kornman *et al.*, 1997; Laxman & Annaji, 2008). A DP é uma doença infecciosa, iniciada por bactérias gram-negativas, e caracterizada pelo acúmulo de células inflamatórias nos tecidos periodontais (Page *et al*., 1997). De acordo com a Academia Americana de Periodontia (2005), 50% dos adultos apresentam pelo menos uma forma moderada de DP. No Brasil, dados do Ministério da Saúde mostraram que há aproximadamente 50% da população entre 35 e 44 anos com DP (Ministério da Saúde, Brasil, 2004).

Tem sido sugerido que, em lesões periodontais, o balanço entre a expressão de citocinas Th1 e Th2 seja um fator relevante da resposta imuno-inflamatória sobre os efeitos da doença (Seymour & Gemmel, 2001; Garlet *et al*., 2003). De fato, o desbalanço dos fatores que regulam a homeostasia dos tecidos conjuntivo e ósseo resulta em desequilíbrio periodontal (Garlet *et al*., 2004). A reação inflamatória é capaz de levar à destruição periodontal como conseqüência do desequilíbrio na expressão das MMPs *versus* (*vs.*) TIMPs, que agem na regulação do *turnover* da MEC, e entre o fator nuclear ativador do receptor-κB (RANK)/fator nuclear ativador do receptor-κB ligante (RANKL) *vs.* osteoprotegerina (OPG), que controlam a ativação e diferenciação osteoblástica/osteoclática (Taubman *et al*., 2005; Hannas *et al*., 2007). Considerando especificamente as MMPs como as mais importantes enzimas envolvidas na destruição do tecido conjuntivo, elas degradam colágeno tipo I, o maior componente estrutural do ligamento periodontal e da matriz orgânica do osso alveolar (Birkedal-Hansen, 1993). Como conseqüência, tem-se o estabelecimento da DP destrutiva (Page *et al*., 1997). Níveis de transcrição das MMPs foram significativamente maiores no tecido gengival de pacientes com DP, comparados a pacientes saudáveis (Kubota *et al*., 1996; Kubota *et al*., 2008). Análises morfométricas de tecido gengival em sítios com DP mostraram aumento nos níveis das MMPs. Além disso, a quantidade e atividade das MMPs tanto no fluido quanto no tecido gengival foram maiores em áreas com DP do que em áreas saudáveis (Soell *et al*., 2002).

Uma emergente gama de evidências tem sugerido que a inflamação decorrente da infecção bucal, em particular a DP, pode não estar restrita ao meio bucal, mas sim, provocar efeitos sistêmicos (Persson & Persson, 2008; Mealey &, Rose, 2008).

Doença Renal Crônica

A doença renal crônica (DRC) é uma desordem inflamatória progressiva, caracterizada pela destruição das unidades funcionais dos rins (néfrons) e resulta de um profundo desequilíbrio hidroeletrolítico, metabólico e imunológico, associado a inúmeras complicações sistêmicas (Proctor *et al*., 2005). A DRC pode ser o resultado de várias doenças como diabetes, hipertensão, glomerulonefrite e desordens autoimunes (De Rossi & Glick, 1996; Proctor *et al.*, 2005; Levey *et al.*, 2007). Ela é classificada em cinco estágios, de acordo com o aumento de sua severidade (*National Kidney Foundation*, 2002; Marakoglu *et al.*, 2003). Independentemente de sua etiologia, a DRC pode progredir para o estágio mais avançado, chamado doença renal em estágio 5, no qual predominam sinais e sintomas de uremia (síndrome urêmica), classificada como insuficiência renal crônica terminal (IRCT). A presença da IRCT indica a necessidade de terapias de substituição da função renal, como a diálise, ou mesmo o transplante renal (*National Kidney Foundation*, 2002; Marakoglu *et al.*, 2003). Nos Estados Unidos, o número de indivíduos com falência renal, tratados por meio de diálise ou transplante aumentou de 209 mil em 1991 para 472 mil em 2004, e a expectativa é de que ultrapasse 650 mil indivíduos até 2010 (Hsu *et al.*, 2004; Coresh *et al*., 2003; Coresh *et al.*, 2007; Schoolwerth *et al.*, 2006). A prevalência da DRC foi de 19,2 milhões nos Estados Unidos (Schoolwerth *et al.*, 2006), e a incidência vem aumentando nos últimos anos. Isso se deve, provavelmente, a uma maior sobrevivência e adesão destes pacientes renais aos programas de tratamento (Coresh *et al.*, 2003). O remodelamento da MEC é o evento-chave na progressão da doença renal. Esta doença resulta de um processo no qual há um desequilíbrio entre o aumento da síntese e a diminuição da degradação dos componentes da MEC, principalmente por ação das MMPs, que por sua vez, estão sob o controle dos TIMPs. Nos rins, as MMPs desempenham um importante papel, pois elas clivam componentes da MB, especialmente o colágeno tipo IV (Ronco *et al.*, 2007). Além disso, o dano à MB é o maior evento da glomerulonefrite. Por outro lado, o excessivo acúmulo de MEC visto na fibrose renal é proveniente da combinação de uma sobreprodução e deficiente degradação dos componentes da MEC (Ronco *et al.*, 2007). Entretanto, considerando-se a multiplicidade de suas ações e a complexidade de sua regulação, os efeitos resultantes da ação das MMPs podem ser diferentes, até mesmo opostos, durante as diversas fases de evolução das nefropatias. Monócitos ativados produzem citocinas, tais como IL-6 e IL-1β, as quais estão aumentadas em pacientes submetidos à hemodiálise (Momoi *et al*., 1995; Yamaguchi *et al*., 1996).

Pacientes renais apresentam altos níveis de IL-6, IL-1*β* e TNF-α, que são fortemente associados a complicações da DRC (Kimmel *et al*., 1998). A expressão excessiva ou inapropriada das MMPs tem sido associada a complicações da DRC, tais como a injúria renal progressiva, a esclerose glomerular (Davies *et al*., 1992; Akiyama *et al*., 1997), fibrose renal intersticial (Norman & Lewis, 1996) e doenças cardiovasculares (Galis *et al.*, 1994; Bini *et al.*, 1996; Ye *et al.*, 1998; Shapiro, 1998).

A DP tem sido considerada como um fator complicador da DRC (Borawski *et al*., 2007; de Souza *et al.*, 2007; Craig, 2008) e foi sugerido que sua prevalência e severidade estão aumentadas nos pacientes renais (Kshirsagar *et al*., 2005). A DP constitui uma significante fonte de inflamação em pacientes renais, tornando a boca um nicho de especial interesse no manejo desses indivíduos (Craig *et al*., 2002; Craig, 2008).

MMP-1 *vs.* DP e DRC

A MMP-1 é uma colagenase produzida por fibroblastos, queratinócitos, células endoteliais, macrófagos, osteoblastos e condrócitos (Birkedal-Hansen, 1993). Elas são secretadas como pró-enzimas inativas (zimógenos) e sua ativação ocorre no tecido através da clivagem da porção pró-peptídeo aminoterminal por outras proteinases (Murphy & Knäuper, 1997). A MMP-1 é a principal enzima proteolítica que pode clivar os colágenos intersticiais nativos tipos I e III, os quais são os componentes protêicos mais abundantes da MEC periodontal. O gene da *MMP1* está localizado no braço longo do cromossomo 11, na posição 22 (11q22) (Pendas *et al*., 1996). Ele é traduzido por uma variedade de células, tais como, fibroblastos, macrófagos, células endoteliais e epiteliais (Birkedal-Hansen *et al*., 1993b; Shin *et al*., 2002; Bar-Or *et al*., 2003). Polimorfismos na região promotora do gene da *MMP1* humano já foram descritos (O-charoenrat *et al*., 2006; Barlas *et al*., 2009). A inserção/deleção de uma guanina (G) na posição -1607 do gene da *MMP*1 humano cria dois diferentes alelos: um alelo contendo apenas uma guanina (1G) e outro, com duas guaninas (2G) (Rutter *et al*., 1998). Foi observado que o polimorfismo 1G/2G é funcional porque aumenta significativamente a atividade de transcrição da MMP-1 (Rutter *et al*., 1998). A presença do alelo 2G foi associada com câncer ovariano (Rutter *et al*., 1998; Kanamori *et al*., 1999) e com carcinoma renal (Hirata *et al*., 2004).

Enquanto para doenças renais crônicas não há relatos de associação com o polimorfismo no gene da *MMP1*, o alelo 2G do polimorfismo *MMP1*-1607 foi associado à DP severa em algumas populações (Souza *et al*. 2003; Cao *et al*., 2006). No

entanto, não há nenhum estudo investigando a associação do polimorfismo no gene da *MMP1* com a DP em pacientes com DRC.

Proposição

PROPOSIÇÃO

Objetivo geral

O objetivo do presente trabalho foi investigar a associação entre polimorfismo no promotor do gene da MMP-1 (*MMP1*-1607, 1G/2G) e a suscetibilidade à DP e à DRC, bem como comparar o nível de transcritos entre os grupos.

Objetivo específico 1

Investigar a associação entre o polimorfismo gênico *MMP1*-1607 (1G/2G) e a suscetibilidade à DP e à DRC, por meio da Reação em Cadeia da Polimerase (PCR) e análise de Polimorfismo de Comprimento de Fragmento de Restrição (RFLP).

Objetivo específico 2

Comparar os níveis de transcritos do gene *MMP1* entre os grupos, por meio de PCR em tempo real, e de acordo com os genótipos.

Artigo

Association between matrix metalloproteinase-1 promoter polymorphism and transcript levels with periodontal disease and chronic kidney disease in a Brazilian population

Sônia Mara Luczyszyn[1], MS; Cléber Machado de Souza[1], DDS; Ana Paula Ribeiro[1], MS; Acir Dirschnabel[1], MS; Fábio Rueda Faucz[2], DDS, PHD; Marcela Claudino[3], DDS, MS; Carlos E. Repeke[3], DDS, MS; Gustavo P. Garlet[3], DDS, PHD; Sung H. Kim[2], DDS, PHD; Vinícius A. Tramontina[2], DDS, PHD; Vula Papalexiou[2], DDS, PHD; Miguel Carlos Riella[4], MD, PHD; Roberto Pecoits-Filho[2], MD, PHD; Paula Cristina Trevilatto[2], DDS, PHD.

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

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

[3] Department of Biological Sciences, School of Dentistry of Bauru, University of São Paulo (USP), Bauru, São Paulo, Brazil.

[4] 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.

Short title: Association between MMP-1 promoter polymorphism with periodontitis and chronic kidney disease.

Key words: periodontal disease; chronic kidney disease; MMP-1; genetic polymorphisms; transcript levels.

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

Objective: Chronic periodontal disease (PD) is an infectious illness of the oral cavity, characterized by inflammatory cell accumulation in the periodontal tissues. The bacteria activate inflammatory mechanisms promoting extracellular matrix (ECM) destruction of the supportive periodontal tissues. Chronic kidney disease (CKD) is an inflammatory progressive disorder characterized by the destruction of the kidneys' functional units (nephrons), which can result from a wide spectrum of diseases such as hypertension and diabetes. Renal patients show higher prevalence of PD and its pronounced progression in these patients can be considered a complication of CKD. Bacteria are essential to PD initiation; however, there are other factors that may not cause the disease, but amplify some progression and severity mechanisms such as tabagism, psychosocial stress, systemic diseases (*e.g.* CKD) and genetic polymorphisms. Polymorphisms are genetic variations frequently found in the population. There are polymorphisms that can influence the activity of regulator factors of inflammatory response, promoting tissue destruction by matrix metalloproteinases (MMPs) action. MMP-1 participates on EMC turnover, and a desequilibrium between its synthesis and degradation may result in tissue destruction, as observed in inflammatory diseases. Higher levels of MMP-1 were found in both fluid and gingival tissues from PD patients, and in both blood and tissues from CKD patients. Higher or inappropriate MMP expression has been associated with CKD complications and PD progression. *MMP1*-1607 (1G/2G) is considered a functional polymorphism as it can alterate MMP-1 expression rate. Thus, the aim of this study was investigate the association of *MMP1*-1607 (1G/2G) polymorphism and susceptibility to PD and CKD.

Material and Methods: The study population consisted of 254 individuals divided into 4 groups: *Group 1*, individuals without PD and without CKD (n=67); *Group 2*, with PD and without CKD (n=60); *Group 3*, without PD and with CKD under hemodialysis (n=52), and *Group 4*, with PD and with CKD under hemodialysis (n=75). Polymorphism *MMP1*-1607 was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). *MMP1* gene transcripts from gingival tissues were analyzed by real time-PCR technique.

Results: It was not observed evidence for association between *MMP1*-1607 polymorphism and PD or CKD. In the expression evaluation, increased levels of transcripts of *MMP1* gene were observed in PD patients gingival tissues. This expression was higher in CKD patients, but the difference was not statistically significant. Within the groups, it was not observed differences in the transcript levels according to genotypes; althought in the presence of 2G allele a progressive increase of *MMP1* expression was noted.

Conclusions: It was concluded that *MMP1*-1607 polymorphism was not associated with PD or CKD. However, higher levels of *MMP1* gene transcripts were found in gingival sites with PD.

Key words: periodontal disease; chronic kidney disease; MMP-1; genetic polymorphisms; transcript levels.

Introduction

Matrix metaloproteinases (MMPs) represent a family of dependent metal ion endopeptidases that is capable of degrading all extracellular matrix (ECM) components (Birkedal-Hansen, 1993; Nagase & Woessner, 1999). They are classified according to the substrate specificity into colagenases, gelatinases, stromelysins, and membrane-bound type (Birkedal-Hansen, 1993; Birkedal-Hansen, 1995). MMP expression is regulated by cytokines; while T-helper type 1 (Th1) and inflammatory mediators interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ have been described as positive regulators of MMPs expression, the reverse effect is exerted by the Th2-type cytokines such as IL-4, IL-10 and IL-13 (Harris *et al*., 2002; Katagiri & Takahashi, 2002). The activation of MMPs is regulated by a group of endogenous proteins named tissue inhibitors of metalloproteinases (TIMPs) that are each able to inhibit almost every member of the MMP family in a non-specific way (Baker *et al.*, 2002). MMPs play an important role in physiological events during embryonic development, morphogenesis, angiogenesis and tissue repair (Sorsa *et al*., 1995; Uitto *et al*., 2003), and is overexpressed in several diseases such as ovarian cancer (Adley *et al*., 2009), atherosclerosis (Romero *et al*., 2008) and osteoarthritis (Hulejová *et al*., 2007). By their ability to degrade ECM, MMPs are at the crossroads of several diseases progression and regression.

Periodontal disease (PD) is a multifactorial disease, in which the susceptibility, progression and outcome are dependent on multiple environmental and genetic factors (Kornman *et al.*, 1997; Laxman & Annaji, 2008). It is a highly prevalent infectious illness of the oral cavity initiated by gram-negative bacteria, characterized by inflammatory cell accumulation in the periodontal tissues (Page *et al*., 1997), which affects at least 50% of adults. It has been suggested that, in periodontal lesions, the balance between the expression of Th1- and Th2-type cytokines in a mixed inflammatory immune response is a relevant factor to the outcome of disease (Seymour & Gemmel, 2001; Garlet *et al*., 2003). The inflammatory reaction is thought to trigger periodontal tissue destruction as a consequence of an imbalance in the expression between MMPs versus (*vs.*) TIMPs, which act regulating the turnover of extracellular and bone matrix degradation (Taubman *et al*., 2005; Hannas *et al*., 2007). The transcriptional levels and activity of MMPs were significantly higher in gingival tissues of individuals with PD when compared to healthy patients (Kubota *et al*., 1996; Kubota *et al*., 2008), and as a consequence, a destructive periodontal disease takes place (Page *et al*., 1997).

An emerging body of evidence suggests that oral inflammatory diseases, and in particular periodontal infections, may not be limited to the immediate oral environment but can have systemic effects (Persson & Persson, 2008; Mealey &, Rose, 2008).

Periodontitis has been considered as a complicator for several systemic diseases such as chronic kidney disease (Borawski *et al*., 2007; de Souza *et al.*, 2007; Craig, 2008), and its prevalence and severity are suggested to be increased in this population (Kshirsagar *et al*., 2005). Destructive PD may be a significant source of inflammation in systemic compromised patients, when periodontal evaluations are not performed as part of a medical assessment (Craig, 2002).

Chronic kidney disease (CKD) is a progressive disorder characterized by the destruction of the kidneys' functional units (nephrons) and it results from a profound hydroelectrolytical, metabolic and immunological imbalance, associated with a number of systemic complications (Proctor *et al*., 2005). CKD can result from a wide spectrum of diseases such as diabetes, hypertension, glomerulonephritis, and autoimmune disorders (De Rossi & Glick, 1996; Proctor *et al.*, 2005; Levey *et al.*, 2007). Kidney disease is divided into five stages of increasing severity (National Kidney Foundation, 2002; Marakoglu *et al.*, 2003). 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). Remodeling of ECM is a key event in progression and reversal of kidney disease. CKD results from a process in which there is a desequilibrium between the increased synthesis of ECM components and decreased ECM degradation mostly by MMPs that are under the control of TIMPs. In the kidney, MMPs are assumed to be important players because they cleave basement membrane (BM) components, mostly type-IV collagen (Ronco *et al.*, 2007). Indeed, BM damage is a major event in crescentic glomerulonephritis. On the other hand, the excessive matrix accumulation, seen in fibrotic kidney, results from a combination of overproduction and defective degradation of matrix components (Ronco *et al.*, 2007). However, considering the multiplicity of their targets and the complexity of their regulation that account for a variety of biological effects, MMP-resulting effects may be different and even opposite during the different phases of the evolution of nephropathies. As described to PD, altered cytokine production may result in disturbance of MMP/TIMPs balance (Kimmel *et al*., 1998). Indeed, excessive or inappropriate expression of MMPs has been associated with CKD complications such as progressive renal injury, glomerular sclerosis (Davies *et al*., 1992; Akiyama *et al*., 1997), intersticial kidney fibrosis (Norman & Lewis, 1996), and cardiovascular diseases (Galis *et al.*, 1994; Bini *et al.*, 1996; Ye *et al.*, 1998; Shapiro, 1998).

MMP-1 is a colagenase produced by fibroblast, keratinocytes, endothelial cells, macrophages, osteoblasts and chondrocytes (Birkedal-Hansen, 1993). It is secreted as an inactive pro-enzyme (zymogen) and its activation occurs in the tissue by cleavage of

the N-terminal pro-peptide domain by other proteinases (Murphy & Knäuper, 1997). The MMP-1 is the major proteolytic enzyme that can cleave native interstitial collagens type I and III, which are the most abundant protein components of periodontal extracellular matrix. Therefore, variance in MMP-1 transcription levels may be relevant to the progression of both PD and CKD. The *MMP1* gene is located in 11q22 (Pendás *et al*., 1996) and present some functional polymorphisms in the promoter region (O-charoenrat *et al*., 2006; Barlas *et al*., 2009). An insertion/deletion of a guanine at position -1607 of the human *MMP*1 gene creates two different alleles: one having a single guanine (1G) and other having two guanines (2G) (Rutter *et al*., 1998). It has been shown that the 1G/2G polymorphism is functional because significantly increases the transcriptional activity of MMP-1 (Rutter *et al*., 1998). The presence of allele 2G was seen to be associated with ovarian cancer (Rutter *et al*., 1998; Kanamori *et al*., 1999), renal carcinoma (Hirata *et al*., 2004), and PD (de Souza *et al*., 2003; Cao *et al*., 2006).

While for chronic renal diseases there are no studies investigating the association with *MMP1*-1607 gene polymorphism, it has been suggested that *MMP1*-1607*2G allele is associated with severe chronic periodontitis in some populations (de Souza *et al*., 2003; Cao *et al*., 2006). Moreover, there is no study investigating the association of *MMP1* gene polymorphism with PD in CKD patients. Thus, the aim of the present work was to analyze the association between polymorphism in the *MMP1*-1607 gene and *MMP1* transcript levels with PD and CKD.

Material and Methods

Study population

A convenient sample of 254 unrelated patients, both sexes, mean age 44.6 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). Although the study sample was mostly composed by Caucasian, the Brazilian white population is heterogeneous. Recent article has not suggested grouping Brazilians into ethnic groups based on color, race and geographical origin because Brazilian individuals classified as white or black have significantly overlapping genotypes, probably due to miscegenation (Parra *et al*., 2003). According to the Brazilian Government Census (2005), in the Brazilian Southern region, the prevalence of white is 77.8%, black, 2.2%, mullatto, 18.9%, and Japanese, 1.1%. Reporting the white population, there is a predominance of Italian, Spanish, and Portuguese heritage. 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 4 groups:

Group 1: 67 individuals without CKD (glomerular filtration rate>90 mL/min estimated according to the Modification of Diet in Renal Disease formula (Levey *et al.*, 1999) and without PD;

Group 2: 60 patients without CKD and with PD [clinical attachment level (CAL)>5 mm, in at least 3 teeth, in at least 2 quadrants];

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

Group 4: 75 patients with CKD stage 5, in hemodialysis and with PD.

Patients presenting 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 PD for groups 1 and 3); use of orthodontic appliances, and present necrotizing ulcerative gingivitis, and PD were not included.

Clinical parameters of PD

Diagnosis of PD was made on the basis of clinical parameters, such as probing pocket depth (PPD) and assessment CAL. Measurements of PPD and CAL were recorded at 4 points around each tooth. Subjects with CAL>5 mm, in at least 3 teeth, in at least 2 quadrants, were considered affected (Armitage, 1999). The following parameters were recorded: the gingival index (GI) (Löe & Silness, 1963); the plaque index (PI) (Silness & Löe, 1964); the calculus index (CI) (Greene & Vermillion, 1964), and mobility (absent or present). The periodontal status of all subjects is shown in table 2.

Clinical parameters of CKD

The following blood cells and serum markers were measured according to the routine of dialysis clinics for CKD patients: C-reactive protein, creatinin,, protein catabolic rate, calcium X phosphorus, KTV (a marker of dialytic adequacy), percentual of reduction urea, urea, potassium, hematocrit, alanine aminotransferase (ALT), albumin, iron, glucose, calcium, phosphorus, ferritin, transferrin, iron saturation index, hemoglobin, alkaline phosphatase, leukocytes, aluminium.

DNA collection and Purification

Cells were obtained through a mouthwash with 3% glucose solution and scraping of the oral mucosa with a sterile spatula (Trevilatto & Line, 2000). DNA was extracted from epithelial buccal cells with ammonium acetate 8 M and EDTA 1 mM (Trevilatto *et al*., 2003; Aidar & Line, 2007).

Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP)

The following primer pair was used for PCR amplification of genomic DNA samples: (F: 5'-TCG TGA GAA TGT CTT CCC ATT-3' and R: 5'-TCT TGG ATT GAT TTG AGA TAA GTG AAA TC-3'). Reaction conditions and cycling parameters were as follows: 1 µL of the genomic DNA was used for PCR amplification in a reaction mixture containing 12.5 µL Go Taq Green Master Mix (Promega, USA), and 1 µL of each 25 µM primer. The reactions were performed in a Techne T-512 thermal cycler and consisted of an initial denaturation step of 95ºC for 5 min, followed by 35 cycles of 95ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min, and a final extension of 72ºC for 7 min.

A 10-µL aliquot of PCR products was mixed with a solution containing 2 µL 10x NE Buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1mM dithiothreitol, pH 7,9), 0.02 µL BSA – bovine serum albumin (10 mg/mL), 0,3 µL XmnI (20 units/mL) (New England Biolabs, Inc., Beverly, MA, USA) and 8 µL sterile deionized H_2O. The solution was incubated at 37°C overnight. Two mismatches were introduced in the reverse primer annealed to the proximity of the polymorphism (Dunleavey *et al.*, 2000), creating a recognition sequence (5'-GAANNNNTTC-3') for the restriction endonuclease X*mn*I, when the DNA template contains 1G (but not 2G) at the polymorphism site. Thus, X*mn*I digests the 1G allele creating two fragments of 89 bp and 29 bp.

The total amount aliquot of the digest was electrophoresed on a 10% vertical non-denaturing polyacrylamide gel at 30 mA. The gel was silver stained with DNA Silver Staining Kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Analysis of cytokines mRNA expression by Real-Time PCR

The pattern of mRNA expression for MMP-1 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 each biopsy represented extreme phenotypes (groups 1 and 3: healthy sites, with no signs of inflammation and attachment loss; groups 2 and 4, presenting bleeding and suppuration, and clinical attachment loss of at least 5 mm).

The extraction of total RNA from periodontal tissues samples, performed with Trizol reagent (Invitrogen), and the cDNA synthesis were accomplished as previously described (Claudino *et al.,* 2008).

Real-time-PCR quantitative mRNA analyses were performed in a MiniOpticon system (BioRad, Hercules, CA, USA) using the SYBR-green fluorescence quantification system (Applied Biosystems, Warrington, UK) for quantitation of amplicons as previously described (Claudino *et al*., 2008). The standard PCR conditions were 95℃ (10 min), and then 40 cycles of 94℃ (1 min), 56℃ (1 min) and 72℃ (2 min), followed by the standard denaturation curve. Real-Time-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: *MMP1* sense TGGACCTGGAGGAAATCTTGC, anti-sense AGAGTCCAAGAGAATGGCCGA; *β*-actin: sense ATGTTTGAGACCTTCAACA; anti-sense CACGTCAGACTTCATGATGG; as previously described (Garlet *et al*., 2004), and 2.5 ng of cDNA were used in each reaction. For mRNA analysis, the relative level of gene expression was calculated in reference to *β*-actin using the cycle threshold (Ct) method. The threshold for positivity of Real-Time-PCR was determined based in negative controls (reactions performed without RNA and without reverse transcriptase). The mean Ct values from duplicate measurements were used to calculate expression of the target gene, with normalization to an internal control (*β*-actin), using the 2-ΔCt formula.

Statistical Analysis

The significance of the differences in observed frequencies of 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. Statistical analysis was performed using statistical software BioEstat 2.0 for Windows and SPSS (Statistical Package for the Social Sciences) 10.0 for Windows (SPSS Inc, Chicago, IL). The risk associated with genotypes, alleles was calculated as the odds ratio (OR) with 95% confidence intervals (CI). For continuous variables, T-student test was used to compare means from 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), followed by Tukey's test. Normality condition of the variables in each group was evaluated using Shapiro-Wilks 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 analysis

The genotype distribution for *MMP1*-1607 polymorphism was consistent with the assumption of Hardy-Weinberg equilibrium.

There was no significant difference (*NS*) in the genotype distribution between the groups for *MMP1* polymorphism (p=0.348), neither was observed statistically significant difference (*SSD*) in the allele frequency for the polymorphism studied (p=0.397). Genotypic frequencies and allelic distribution are shown in table 3. When group 1 (control) was examined versus (*vs*.) group 2 (patients with PD), *NS* was noted for the genotypic (p=0.335) and for allelic (p=0.956) distributions. The same was verified between group 1 (control) and group 3 (patients with CKD) for genotypic (p=0.145) and allelic (p=0.212) frequencies, and between group 1 (control) and group 4 (patients with CKD and PD) for genotype (p=0.582) and allele (p=0.359) distributions.

Genotyping and PD clinical parameters

Regarding the clinical periodontal status, *MMP1*-1607*1G allele was associated to higher means of PI in groups 1 and 2 (p=0.045). It was observed an association of 1G allele with PPD (p=0.053) and CAL (p=0.042) for CKD patients (groups 3 and 4).

Genotyping and CKD *clinical parameters*

Considering CKD patients (groups 3 and 4), higher levels of serum markers were associated with *MMP1**2G allele: alanine aminotransferase (ALT) (p=0.026) and calcium (p=0.014). *MMP1**1G allele was associated to higher levels of ferritin (p=0.030).

In relation to clinical aspects of CKD, there was an association (p=0.003) between *MMP1**2G allele and hepatitis.

Quantitative analysis of MMP-1 mRNA expression

There was an augment in the expression of the *MMP1* gene in groups with PD (groups 2 and 4). Although the levels of expression were higher in group 4 in comparison with group 2, *SSD* was not observed (Fig. 1). A *NS* was observed between *MMP1* gene expression and the periodontal parameters.

In relation to *MMP1*-1607 genotype distribution in the whole sample, no *SSD* was observed. In group 4, there was an increase in the *MMP1* transcripts in the presence of 2G allele, but equally *NS* was observed (Fig. 2).

Discussion

Matrix metalloproteinases play an important role in both degradation and remodeling of ECM proteins during different physiological and pathological processes (Birkedal-Hansen *et al.*, 1993b). In physiological conditions, TIMPs are in balance with the MMPs and the ECM is remodeled in a highly regulated fashion. However, in many cases the levels of MMPs are elevated without a concomitant increase in TIMPs, resulting in tissue destruction, as seen in some inflammatory diseases, *e.g.,* PD (Garlet *et al*., 2004; Kubota *et al*., 2008) and CKD (Han *et al*., 2006; Sharma et al., 1995; Ebihara *et al*., 1998; Preston *et al*., 2002).

In the present study, *NS* was observed in the genotype distribution neither was observed *SSD* in the allele frequency for *MMP1*-1607 polymorphism among the groups. *MMP1*-1607 is a functional polymorphism and there is a small number of studies investigating its association with PD (de Souza *et al*., 2003; Hollá *et al*., 2004; Itagaki *et al*., 2005; Astolfi *et al*., 2006; Cao *et al*., 2006; Ustun *et al*., 2008). This polymorphism was investigated for chronic periodontitis in Asiatic populations. It was not observed any association of the study polymorphism with chronic periodontitis in Japanese patients, although 2G/2G genotype, 2G allele frequency and 2G carriage rate were increased in severe chronic periodontitis (Itagaki *et al*., 2004). Moreover, the frequency of 2G allele and 2G/2G genotype was higher in subjects with severe periodontitis in a Chinese population (Cao *et al*., 2006). There has been some controversy in the results on the analysis of this polymorphism regarding Brazilian populations. Astolfi *et al*. (2006) observed no association of genotype and allele distribution between healthy and PD groups. On the other hand, a previous study had reported an association of *MMP1**2G allele with severe chronic periodontitis (de Souza *et al*., 2003). More recently, two studies in Turkish populations showed different results; Pirhan *et al*. (2008) evidenced an association of *MMP1**2G allele with severe periodontitis and no association was found of this allele with periodontitis in the study by Ustun *et al*. (2008). These discrepancies may reflect the involvement of variable combinations of risk alleles in different populations, confounding factors such as genetic admixture, regional differences in gene frequencies and gene-environment interactions, and a poor sample selection of extreme phenotypes (Hollá *et al*., 2004). In fact, we observed that most studies considering extreme phenotypes were able to detect an association between *MMP1*-1607 gene polymorphism and chronic

periodontitis (de Souza *et al*., 2003; Itagaki *et al*., 2004; Cao *et al.*, 2006, Pirhan *et al*., 2008). Thus, these data may indicate that *MMP1*-1607 gene polymorphism is associated to PD severity rather than susceptibility.

Interestingly, in regards of periodontal indexes, we found that *MMP1**1G was associated with PI in groups without and with PD. Based on the presence of both periodontal bacteria and interleukins (*e.g*., IL-1, IL-6), which regulate the MMP-1 expression, it would be expected to detect the association of *MMP1**2G allele with higher means of PI, however dental biofilm represents further a favorable environment for the initiation than the progression of PD. Disease progression and severity may be further dependent on the immune-inflammatory host response to mibrobial challenge (Garlet *et al*., 2003). *MMP1**1G was also associated with higher means of PPD and CAL in CKD patiens. The gene expression level for MMP-1 seems to be up-regulated in periodontitis tissues compared to non-periodontitis samples. Indeed, *MMP1* mRNA expression levels have been found to be increased in periodontitis-affected gingival tissue (Garlet *et al.*, 2004; Kubota *et al*., 2008). The components of the periodontal tissue extracellular matrix, especially collagens, appear to be the main targets of degradation in periodontitis. Among host proteases degrading the ECM, MMPs seem to be highly related to tissue destruction and remodeling events in PD (Reynolds *et al*., 1994, Van der Zee *et al*., 1997).

In this work, it was used real-time PCR to evaluate the expression of *MMP1* transcripts in patients presenting or not PD and CKD. The levels of *MMP1* transcripts were higher in group with PD and CKD in comparison with group with only PD, but *NS* was observed, probably by the circumstances of the presence of a systemic inflammatory condition. Moreover, in the group with both diseases, there was a progressive increase of *MMP1* transcripts in the presence of allele 2G. It was previously described that an additional guanine (G) creates an Ets (a family of transcription factors) binding site, promoting a significantly increased transcription in normal fibroblasts, providing a mechanism for more aggressive matrix degradation (Rutter *et al*., 1998). Maybe, in the presence of a systemic disease, the association of allele 2G with the augment of gene expression in PD is more evident. Furthermore, it was noted the presence of a high variation in the *MMP1* expression between individuals having the same genotype in groups with PD. This could be due to the additional influence of specific periodontopathogens and cytokines stimulation.

To our knowledge, this is the first study carried on to investigate the association of *MMP1*-1607 polymorphism with CKD. In this study, no difference was observed in the frequency of genotypes and alleles between renal and control patients. However, other polymorphisms in *MMP* genes may have an impact in response to the CKD

inflammatory insult. Excessive or inappropriate expression of MMPs has been associated with progressive renal injury, glomerular sclerosis (Davies *et al*., 1992; Akiyama *et al*., 1997) and interstitial kidney fibrosis (Norman & Lewis, 1996). Preston *et al.* (2002) indicated that there are measurable differences in the expression of MMPs within the dialysis patient population. Because dialysis can be associated with local and systemic inflammation, increased levels of certain MMPs in hemodialysis groups may be a reflection of gene stimulation induced by inflammatory cytokines. It was found an association between *MMP1**2G allele and both higher levels of ALT and hepatitis. The transaminases, such as ALT, are considered as "classical" serum markers of liver necro-inflammatory injury (Holoman *et al*., 2002). It is recommended that patients on hemodialysis therapy, which constitute a risk group for hepatitis C virus (HCV) infection, are submitted to regular screening for HCV infection by using ALT values (Saab *et al*., 2001). The HCV infection is frequently asymptomatic in these patients and should be suspected in all patients presenting elevated ALT (Lemos *et al*., 2008). The prevalence of HCV is significantly higher in hemodialysis and kidney transplant patients, as compared to the general population (Baid-Agrawal *et al*., 2008). Chronic HCV infection remains an important cause of liver disease in patients with ESRD and conversely, renal failure has a significant impact on morbidity and mortality throughout the natural history of chronic HCV and its treatment (Okoh *et al*., 2008). Also, *MMP1**1G allele was associated to higher levels of ferritin. Serum ferritin is a clinically important index of body iron stores, but its functional role remains largely obscure (Harrison & Arosio, 1996). Once that ferritin is also considered as an acute-phase reactant (Wish, 2006), inflammatory factors may interfere with the synthesis and clearance of ferritin, thereby increasing serum ferritin levels unrelated to iron status (Kalantar-Zadeh *et al.*, 2004). Higher levels of serum calcium were associated with *MMP1**2G allele. Current clinical guidelines recommend that serum levels of calcium and phosphorus should be maintained within the target range to avoid the development of vascular calcification (National Kidney Foundation, 2003). The kidney plays a leading role in maintaining calcium and phosphorus homeostasis in collaboration with other organs, such as parathyroid gland, intestines, and bones. Along with the progression of CKD, various abnormalities of mineral and bone metabolism may exist (Martin & Gonzalez, 2007). Traditionally, such disorders have been considered with regard to the bone lesion itself, but it has become increasingly evident that mineral and bone disorders have a critical role in the pathogenesis of extraskeletal calcification including the vasculature, thereby resulting in cardiovascular complications and mortality (Moe *et al*., 2006). In addition, recently, it was reported that 2G allele associated with mortality in hemodialysis patients (Cozzolino *et al*., 2009).

Chronic infections such as PD seem to be significant causes of persistent systemic inflammation in patients with CKD (de Souza *et al*., 2007) and, in turn, systemic conditions such as CKD may impact PD outcome (Craig, 2008). Moreover, MMPs are overexpressed in inflammatory conditions such as PD and CKD, increasing the destruction of ECM components. Thus, investigating polymorphisms which interfere with the regulation of MMP expression may contribute for the understanding of common mechanisms of genetic background.

In summary, it was not observed any evidence for association of *MMP1**2G with susceptibility to PD or CKD. It was observed an augment of the levels of *MMP1* transcripts in periodontally affected tissues.

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Table 1. Baseline characteristics of the study population.

	Group 1 (n=67)	Group 2 (n=60)	Group 3 (n=52)	Group 4 (n=75)	*p* value
Ethnic Group n (%)					
Caucasoid	53 (79.1)	46 (76.7)	38 (73.1)	52 (69.3)	0.646 *
Mulatto	10 (14.9)	13 (21.7)	12 (23.1)	18 (24.0)	
Afro-American	4 (6.0)	1 (1.7)	2 (3.8)	5 (6.7)	
Age years (range) †	38.4 ± 9.5 (20 – 70)	41 ± 9.3 (20 – 61)	45.3 ± 2.5 (23 – 74)	53.8 ± 12.1 (26 – 77)	< 0.001 **
Gender n (%)					
Female	49 (73.1)	40 (66.7)	18 (34.6)	26 (34.7)	< 0.001 *
Male	18 (26.9)	20 (33.3)	34 (65.4)	49 (65.3)	

†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**: with PD and without CKD. **Group 3**: without PD and with CKD. **Group 4**: presenting PD and CKD.

Table 2. Periodontal status of the study population.

Periodontal Indexes	Group 1 (n=67)	Group 2 (n=60)	Group 3 (n=52)	Group 4 (n=75)	*p* value
Gingival Index †	0.2 ± 0.4	1.6 ± 1	0.4 ± 0.6	0.8 ± 0.9	<0.001*
Plaque Index †	0.3 ± 0.4	1.3 ± 1	0.6 ± 0.9	1.1 ± 1	<0.001*
Calculus Index †	0.2 ± 0.2	0.9 ± 0.9	0.4 ± 0.8	0.8 ± 0.9	<0.001*
PPD [a] (mm) †	1.5 ± 0.1	2 ± 0.5	1.3 ± 0.6	1.7 ± 0.7	<0.001*
CAL [b] (mm) †	1.5 ± 0.1	2.3 ± 0.9	1.3 ± 0.6	2.4 ± 1.4	<0.001*
Mobility (absence/presence)	65/2	35/24	37/12	37/34	<0.001**

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

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

Table 3. Allelic and genotypic distribution of *MMP1*-1607 (1G/2G) gene polymorphism.

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Chi-square *p* value**
Genotype	N=67 (%)	n=60 (%)	n=52 (%)	N=75 (%)	
1G/1G	18 (26.9)	11 (18.3)	7 (13.5)	13 (17.3)	χ^2=6.71
1G/2G	26 (38.8)	32 (53.3)	26 (50.0)	31 (41.3)	*p*=0.348
2G/2G	23 (34.3)	17 (28.3)	19 (36.5)	31 (41.3)	
Allele	n=134 (%)	n=120 (%)	n=104 (%)	n=150 (%)	
1G	62 (46.3)	54 (45)	40 (38.5)	57 (38)	χ^2=2.96
2G	72 (53.7)	66 (55)	64 (61.5)	93 (62)	*p*=0.397

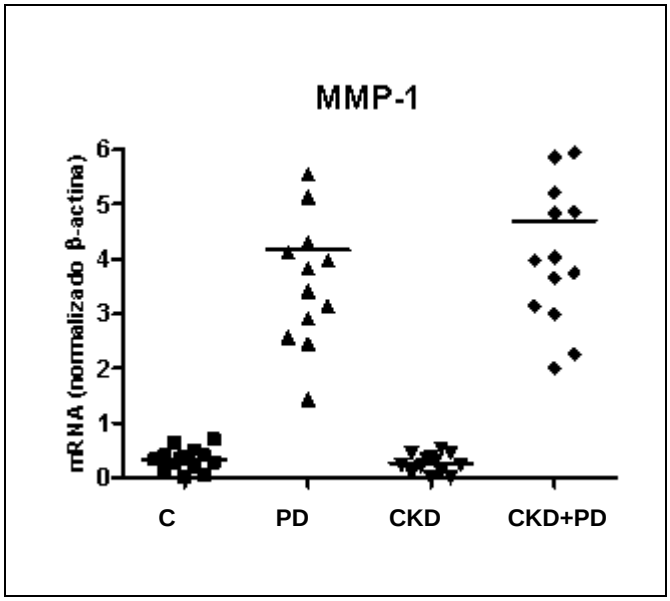

Fig. 1. Quantitative expression of *MMP1* gene in gingival tissues of patients from the four groups. Total RNA was extracted, and levels of *MMP1* were measured quantitatively by real-time PCR SYBR-Green System. The results are presented as the fold increase of expression of the mRNA, with normalization to *β*-actin, when compared with the target-internal control of control subjects using the cycle threshold (Ct) method.

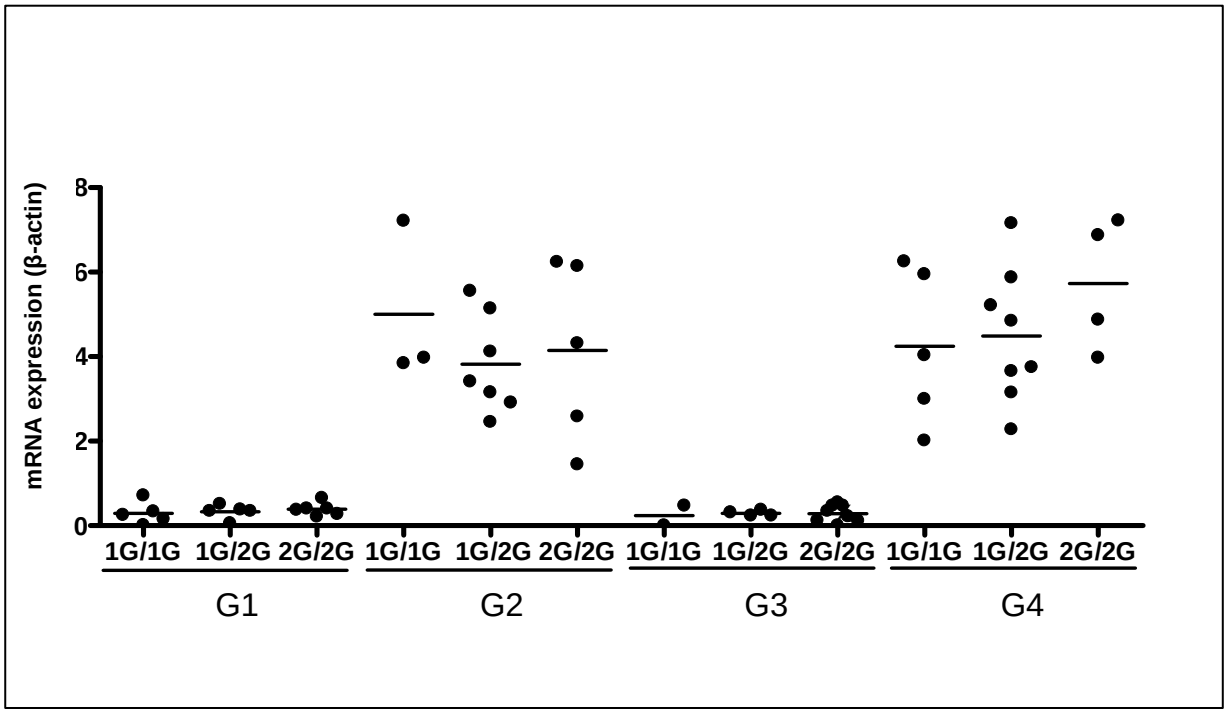

Fig. 2. Quantitative expression of *MMP1* gene in gingival tissues of patients from the four groups according to genotypes 1G/1G, 1G/2G, 2G/2G. Total RNA was extracted, and levels of *MMP1* were measured quantitatively by real-time PCR SYBR-Green System. The results are presented as the fold increase of expression of the mRNA, with normalization to *β*-actin, when compared with the target-internal control of control subjects using the cycle threshold (Ct) method.

Conclusão

CONCLUSÃO

i) Não foi observada evidência de associação do polimorfismo gênico *MMP1*-1607 (1G/2G) com a suscetibilidade genética à DP ou à DRC.

ii) Foi observado um aumento nos níveis dos transcritos do gene *MMP1* em tecidos com DP. Os genótipos do polimorfismo *MMP1*-1607 parecem influenciar a expressão gênica; porém os resultados não apresentaram significância estatística.

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Anexo

Outros artigos publicados relacionados à tese:

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 qui-square 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

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.

ARTIGO 3:

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.