CLEBER MACHADO DE SOUZA

ANÁLISE DA ASSOCIAÇÃO ENTRE POLIMORFISMOS NO GENE DO RECEPTOR DA VITAMINA D (VDR) E A SUSCETIBILIDADE À DOENÇA RENAL CRÔNICA E À DOENÇA PERIODONTAL

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.

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Co-Orientador: Prof. Dr. Roberto Pecoits-Filho

CURITIBA

2007

DEDICATÓRIA

A Deus, que sem a sua sabedoria nada nesse mundo seria possível...

Aos meus pais que são os responsáveis por aquilo que sou.

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RESUMO

A doença renal crônica (DRC) e a doença periodontal (DP) são sérios problemas de saúde-pública, sendo esta última especialmente negligenciada na população de renais crônicos. A vitamina D é um hormônio esteróide solúvel, metabolizado no fígado e nos rins, resultando em sua forma ativa que interage com o seu receptor nuclear (VDR) para regular ampla variedade de processos biológicos, que incluem o metabolismo ósseo, a modulação da resposta imune e a transcrição de genes envolvidos nos mecanismos básicos da DRC e da DP. Nossa hipótese é que polimorfismos que possuem um impacto na expressão do gene do VDR estão associados com a DRC e com a DP. Assim, o objetivo do presente trabalho foi investigar a associação entre polimorfismos no gene do VDR e a suscetibilidade à doença renal crônica e à doença periodontal. Duzentos e vinte e dois (N=222) indivíduos sem e com DRC, em hemodiálise, foram divididos em grupos com e sem DP. Os polimorfismos *Bsm*I e *Taq*I no gene do VDR foram analisados por PCR-RFLP. A significância das diferenças nas freqüências alélicas e genotípicas de cada polimorfismo entre os grupos foi acessada pelo teste qui-quadrado (p<0,05). O risco associado com os genótipos, alelos e haplótipos foi calculado pelo *odds ratio* (OR), com intervalo de confiança de 95 %. Foi observada evidência de associação entre o alelo G do polimorfismo *Bsm*I com a proteção contra a DRC. Nenhuma associação foi observada entre os polimorfismos estudados e a suscetibilidade ou a proteção contra a DP. Foi concluído que o alelo G do polimorfismo *Bsm*I do gene do VDR possui um efeito protetor contra o desenvolvimento da DRC.

Abstract

ABSTRACT

Chronic kidney disease (CKD) and periodontitis (PD) are serious public-health concerns, but the latter has been especially neglected in the CKD population. Vitamin D is a fat-soluble steroid hormone, metabolized in the liver and then in the kidney, resulting in the active form that interacts with its nuclear receptor (VDR) to regulate a wide variety of biological processes including bone metabolism, modulation of the immune response, and transcription of several genes involved in CKD and PD baseline disease mechanisms. Our hypothesis is that polymorphisms, which have an impact in the vitamin D receptor gene expression, are associated with CKD and PD. Thus, the aim of the present work was to investigate the association of polymorphisms in the VDR gene with chronic kidney disease and periodontitis. Two hundred twenty-two (N=222) subjects with and without CKD in hemodialysis were divided into groups with and without PD. Polymorphisms *Bsm*I and *Taq*I in the VDR gene were analyzed by PCR-RFLP. The significance of the differences in allele and genotypic frequencies of each polymorphism between the groups was assessed by standard Chi-square, *p*-value < 0.05. The risk associated with genotypes, alleles and haplotypes was calculated as the odds ratio (OR) with 95 % confidence intervals. It was observed an evidence of association between allele G of *Bsm*I polymorphism and protection against chronic kidney disease. No association was observed between the study polymorphisms and susceptibility to or protection against periodontal disease. It was concluded that allele G of VDR *Bsm*I polymorphism has a protective effect against CKD development.

Introdução

INTRODUÇÃO

Doença Renal Crônica (DRC)

A doença renal crônica (DRC) tem recebido atenção especial nos últimos anos devido ao seu crescimento em todo o mundo. O aumento de pacientes com DRC implica em alto custo de tratamento e qualidade de vida comprometida para os portadores dessa doença. A DRC é uma doença inflamatória progressiva, caracterizada pela destruição das unidades funcionais dos rins, os nefros. Uma vez destruídos, os nefros lesados não se regeneram, promovendo uma hipertrofia dos nefros normais (Riella & Pecoits-Filho, 2003). Estudos epidemiológicos demonstram que, para cada paciente mantido em diálise, devam existir cerca de 20 a 25 pacientes com algum grau de comprometimento renal, ou seja, no Brasil, em torno de 1,2 a 1,5 milhão de pacientes com algum grau de disfunção renal (Atkins, 2005).

Na atualidade, a DRC é definida quando indivíduos apresentam filtração glomerular (FG) menor que 60 mL/min/1,73 m^2, por três meses ou mais (*Kidney Disease Outcome Quality Initiativ*e, 2002; Diretrizes Brasileiras de Doença Renal Crônica, 2004). Indivíduos normais apresentam como função renal o somatório da função de milhões de nefros. Agressões que acarretem a perda irreversível de algumas dessas unidades funcionais resulta em DRC. Portanto, a mesma definição serve para pacientes que perderam, por exemplo, 10 % da função renal global, bem como para aqueles que perderam 90 % dessa mesma função (Barros & Gonçalves, 2007). Apesar das diferenças na progressão da DRC, os resultados finais são múltiplos sinais e sintomas comuns, decorrentes da incapacidade dos rins em manter a homeostasia interna.

Em fase mais avançada da DRC, há a necessidade de reposição da função renal através da diálise, que é um meio artificial de remoção dos produtos tóxicos do metabolismo da corrente circulatória. Com o avanço tecnológico das terapias substitutivas da função renal, a sobrevivência de pacientes renais crônicos se estendeu sobremaneira, e várias complicações passaram a acometer os pacientes renais crônicos.

Uma das principais complicações que acometem os pacientes renais estão relacionadas com fatores ligados ao metabolismo ósseo. Nos pacientes em diálise, a secreção do paratormônio (PTH) é estimulada de maneira persistente em resposta a diversos fatores, tais como redução da função renal, produção diminuída de vitamina D e hipocalcemia. O PTH estimula a ativação de osteoclastos que irão remover o

cálcio da parte mineral do osso e esse cálcio, então, será disponibilizado para a circulação.

Uma das principais causas de mortalidade e de morbidade na população em todo o mundo é a doença cardiovascular (DCV) (Stenvinkel et al., 2003) e os distúrbios do metabolismo mineral têm sido considerados como fatores determinantes na aceleração do processo de calcificação das artérias (Ibels et al., 1977; Davies et al., 2001; Salusky et al., 2002). O risco de DCV é mais acentuado em pacientes com DRC, sendo responsável por 50 % das mortes nestes pacientes (Foley et al., 1998). Fatores tradicionais de risco para DCV, tais como idade, sexo, hipertensão e dislepidemia, não podem explicar o excesso da mortalidade em pacientes renais (Kasiske, 2001). Esta amplificação do risco à DCV em pacientes renais tem sido atribuída a vários fatores de risco emergentes, tais como estresse oxidativo, hiperfosfatemia, calcificação vascular e inflamação crônica (Kitiyakara et al., 2000; Mezzano et al., 2001; Pecoits et al., 2002).

A inflamação sistêmica persistente em pacientes com DRC parece ser causada por infecções crônicas, sendo os pacientes renais crônicos usualmente mais propensos à infecção do que a população em geral (Steinvinkel et al., 2004). Assim, complicações metabólicas, imunológicas e infecciosas têm sido um grande desafio no manejo clínico dos pacientes renais crônicos.

Doença Periodontal (DP)

A doença periodontal (DP) ou periodontite representa um grupo de doenças inflamatórias que afetam os tecidos de suporte dos dentes. A doença periodontal resulta da interação de espécies bacterianas (biofilme ou placa dental) com componentes da resposta imuno-inflamatória do hospedeiro (Armitage, 1999). A reação inflamatória na periodontite pode resultar na formação de uma *bolsa periodontal* ao redor do dente afetado, pela perda do ligamento periodontal e osso alveolar, que caracterizam os tecidos de suporte do dente (periodonto de sustentação).

De acordo com a *American Academy of Periodontology* (2005), 50 % dos adultos têm ao menos a forma moderada de doença periodontal. No Brasil, de acordo com dados do ministério da Saúde (2003) 50 % da população entre 35 e 44 anos apresentam algum tipo de DP.

Aspectos da resposta do hospedeiro à presença bacteriana, que são o elemento-chave do início e progressão da periodontite, podem ser geneticamente determinados (Hart, 1994). A DP tem sido considerada uma complicação na DRC (Borawski et al., 2007) e é sugerido que a sua prevalência e severidade estão aumentadas nessa população (Kshirsagar et al., 2005). A existência de doença periodontal representa foco de infecção aos pacientes renais crônicos, os quais são extremamente suscetíveis a estas (Sowell, 1982).

Vitamina D

A vitamina D é essencial para a homeostasia normal de cálcio e de fósforo, bem como para o desenvolvimento e manutenção do tecido ósseo. As manifestações clínicas da deficiência de vitamina D, por exemplo, o raquitismo, tem sido reconhecido por vários séculos. Em 1919, Sir Edward mostrou que o raquitismo era causado por uma deficiência nutricional de uma substância solúvel. Esta descoberta iniciou a identificação de um composto solúvel com atividade anti-raquítica. Diferentemente de um outro composto descoberto na mesma época (chamado de vitamina A), esse composto foi chamado de vitamina D. A demonstração de que a vitamina D pode ser produzida de forma endógena em humanos indicou que esta substância não era na verdade uma vitamina. Então, em 1932, a elucidação da estrutura química da vitamina D revelava que esta “vitamina” era, de fato, um hormônio esteróide (Dusso & Brown, 1998).

A vitamina D pertence a uma família de esteróides lipossolúveis biologicamente ativos e necessita de metabolização prévia para ser ativada e atuar em tecidos-alvo (Jones, 2007). Dois terços da vitamina D que o corpo humano contém é sintetizado a partir da molécula precursora 7-dehidrocolesterol na pele por ativação da luz do sol e um terço é obtido por meio da ingesta alimentar. Para sua ativação, a vitamina D é hidroxilada na posição 25 pela enzima 25-hidroxilase, convertendo-se em $25(OH)D_3$ no fígado, sendo este o maior metabólito circulante da vitamina D. O hormônio biologicamente ativado $1,25(OH)_2D_3$ é gerado por hidroxilação do produto $25(OH)D_3$. Esta reação ocorre por adição de uma hidroxila na posição um, sendo catalisada pela enzima 25-hidroxivitamina-D_3-1α-hidroxilase, localizada nos túbulos proximais dos rins. O controle estrito das concentrações séricas de $1,25(OH)_2D_3$ é ditado pela necessidade de cálcio do organismo e envolvem ações coordenadas de clássicos

órgãos regulatórios da atividade mineral, como os rins, intestino, osso e glândulas paratireóides (Dusso & Brown, 1998).

Receptor da Vitamina D (VDR)

O receptor da vitamina D (VDR) é uma proteína nuclear membro da superfamília dos receptores hormonais esteroidais que medeia as ações biológicas da vitamina D (Nezbedova & Brtko, 2004). A vitamina D interage especificamente com o VDR e esse complexo associa-se à molécula de DNA no núcleo da célula, promovendo a repressão ou estimulação de determinados genes responsivos à vitamina D (VDRE). Igualmente a outros receptores nucleares hormonais o VDR apresenta vários domínios de ligação e diversos co-fatores que complementam todo o maquinário transcricional (Whitfield et al., 1995).

No homem, a proteína VDR conta com 427 aminoácidos e está presente em mais de 30 tecidos-alvo (Reichel et al., 1989), três deles envolvidos com a homeostasia de cálcio (intestinos, ossos e rins).

Gene do Receptor da Vitamina D (VDR)

O gene do VDR da galinha foi clonado primeiramente (McDonnell et al., 1987) e, posteriormente, o gene do VDR humano (Baker et al., 1988). O VDR humano é um produto do gene localizado no braço longo do cromossomo 12 na posição 13-14 (12q13-14). O gene possui 11 éxons que, junto com os íntrons, mede aproximadamente 75 kb (Miyamoto et al., 1997). Possui uma extensa região promotora capaz de gerar múltiplos transcritos tecido-específicos (Crofts et al., 1998). Camundongos *knockout* para o gene do VDR mostraram hipocalcemia, hipofosfatemia, hipoparatireoidismo, raquitismo e osteomalácia (Sakai & Demay, 2000), demonstrando sua importância na manutenção da homeostasia (Goltzman et al., 2004).

Alterações na seqüência gênica, que geram formas variantes comuns na população, são conhecidas como polimorfismos. Mais de 100 polimorfismos estão presentes no gene do VDR (Uitterlinden et al., 2002) e foram associados com condições patológicas, como câncer de mama (Lundin et al., 1999; Guy et al., 2004), de cólon e reto (Slattery et al., 2004), de próstata (Cheteri et al., 2004), asma (Poon et

al., 2004), diabetes tipo I (Marti et al., 2004), osteoporose (Sun et al., 2002; Duman et al., 2004), osteoartrite (Utterlinden et al., 1997), aspectos de massa e *turnover* ósseos (Karkoska et al., 1998), fraturas osteoporóticas (Langdahl et al., 2000), e doença periodontal (de Brito Jr et al., 2004).

Entre esses polimorfismos, estão incluídos polimorfismos de base única (SNP) evidenciados pelas enzimas de restrição *Bsm*I (Morrison et al., 1992) e *Taq*I (Morrison et al., 1994). Esses SNPs podem alterar a taxa de transcrição do gene do VDR, por estarem em desequilíbrio de ligação (DL) com a região não-traduzida (UTR) 3', especialmente pela modificação de estabilidade do RNAm (Decker & Parker, 1995). Por regular a disponibilidade do VDR, esses polimorfismos são considerados funcionais.

A abundância e a grande freqüência de SNPs no genoma humano os transformam em alvo para explicar a variabilidade genética (Korstanje & Paigen, 2002; Thomas & Kejariwal, 2004) e sua influência no risco e progressão de doenças comuns na população (Morange et al., 2005; Rao et al., 2005).

Polimorfismos no Gene do VDR e a Doença Renal Crônica

Vários estudos têm associado polimorfismos genéticos no gene do receptor da vitamina D (VDR) com a DRC.

O polimorfismo *Bsm*I do gene do VDR foi associado com parâmetros de massa e densidade óssea em pacientes hemodialisados (Akiba et al., 1997; Gómez Alonso et al., 1998; Karkoszka et al., 1998; Giannini et al., 2002; Rubello et al., 2005). No entanto, Kohama et al. (2000) não observaram envolvimento dos polimorfismos *Apa*I, *Fok*I, *Bsm*I e *Taq*I no gene do VDR na determinação de parâmetros ósseos em pacientes em hemodiálise. O polimorfismo *Bsm*I do gene do VDR foi associado a hipercalcemia em pacientes com diálise peritoneal (Akcay et al., 2005). O polimorfismo *Bsm*I também tem sido associado com níveis alterados de hormônio paratireoídeo (PTH), o que pode sugerir uma influência desse polimorfismo na regulação da função da paratireóide em pacientes renais crônicos (Fernandez et al., 1997; Gómez Alonso et al., 1998; Tagliabue et al., 1999; Marco et al., 2001; Giannini et al., 2002; Falkiewicz et al., 2005; Rubello et al., 2005). Yokoyama et al. (1998) observaram associação do polimorfismo *Apa*I, mas não o *Bsm*I, com níveis aumentados de PTH em pacientes em estágio final da DRC. Estes mesmos autores notaram que o polimorfismo *Apa*I do gene do VDR esteve relacionado com a resposta do PTH a dialisados com cálcio

(Yokoyama et al., 2001). O polimorfismo *Fok*I do gene do VDR também foi associado com níveis séricos de PTH (Rendina et al., 2004; Vigo et al., 2005) em pacientes com DRC. O polimorfismo *Bsm*I tem sido também associado ao hiperparatireoidismo secundário, complicação freqüente na DRC, em pacientes em hemodiálise (Nagaba et al., 1998; Tagliabue et al., 1999; Chudek et al., 2000; Borràs et al., 2003) e em pré-diálise (Marco et al., 1999). Marco et al. (2001) observaram que esse polimorfismo influenciou a resposta ao tratamento com o calcitriol e a sobrevida em pacientes hemodialisados. O mesmo polimorfismo mostrou ser o preditor mais importante relacionado com os baixos níveis de hemoglobina e a necessidade de administração de eritropoetina recombinante humana (Ertürk et al., 2002; Sezer et al., 2007), e esteve relacionado com o lupus eritematoso (Ozaki et al., 2000) em pacientes renais crônicos. O polimorfismo *Bsm*I mostrou associação com a piora da função renal em trabalhadores contaminados por chumbo (Weaver et al., 2005; 2006). Os polimorfismos *Bsm*I e *Taq*I mostraram relação com nefrolitíase e formação de pedras nos rins (Mosseti et al., 2004). No entanto, Gunes et al. (2006) concluíram que os polimorfismos *Bsm*I, *Taq*I e *Apa*I do gene do VDR não conferem um significante risco para a urolitíase. O polimorfismo *Taq*I foi associado com diabetes pós-transplante (Numakura et al., em 2005), doença de Crohn (Simmons et al., 2000) e risco de carcinoma renal (Ikuyama et al., 2002) em pacientes renais crônicos. Numa população japonesa, Obara et al. (2007) encontraram associação do polimorfismo *Apa*I do gene do VDR com o risco de incidência e pobre prognóstico do carcinoma renal, mas essa associação não ocorreu para os polimorfismos *Taq*I e *Bsm*I.

Polimorfismos no gene do VDR e a Doença Periodontal

Recentemente, um número de genes da resposta imuno-inflamatória do hospedeiro foi investigado, inclusive na população brasileira, e polimorfismos foram associados à DP crônica (Scarel-Caminaga et al., 2002 a,b; de Souza et al., 2003 a,b; Scarel-Caminaga et al., 2003, Trevilatto et al., 2003 a,b; de Brito Jr, 2004; Scarel-Caminaga et al., 2004).

Especificamente no gene do VDR, o polimorfismo *Bsm*I foi associado com a susceptibilidade ao desenvolvimento da DP agressiva (Yoshihara et al., 2001), que acomete indivíduos precocemente, e DP crônica, geralmente presente em indivíduos a partir de cerca de 25 anos de idade (Tachi et al., 2003; de Brito Jr. et al., 2004; Brett et al., 2005). No entanto, Zhang et al. (2005) não encontraram associação entre alelos e

genótipos dos polimorfismos *Bsm*I *e Taq*I no gene do VDR em população com DP crônica. O polimorfismo *Taq*I também foi associado a um aumento significante no risco de desenvolver periodontite agressiva (Hennig et al., 1999; Tachi et al., 2001; Sun et al., 2002) e crônica (Tachi et al., 2001; de Brito Jr. et al., 2004). Park et al. (2006) encontraram associação entre o polimorfismo *Fok*I e a doença periodontal agressiva e não houve associação para os polimorfismos *Taq*I e *Bsm*I. Essa associação com o polimorfismo *Fok*I também foi evidenciada por Naito et al. (2007) em pacientes japoneses com periodontite crônica. A investigação entre a progressão da DP e os polimorfismos *Taq*I e *Apa*I do gene do VDR em um estudo longitudinal foi realizado por Inagaki et al. (2003), que observaram associação entre o polimorfismo *Apa*I e a perda óssea alveolar, nível de inserção clínica e a perda de dentes.

Proposição

PROPOSIÇÃO

O objetivo do presente trabalho foi investigar a associação entre polimorfismos no gene do receptor da vitamina D (VDR) e a suscetibilidade à doença renal crônica (DRC) e à doença periodontal (DP).

Artigo 1

Artigo 1

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Association between Vitamin D Receptor Gene Polymorphisms and Susceptibility to Chronic Kidney Disease and Periodontitis

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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 *Taq*I and *Bsm*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 $\chi 2$ 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.

Key Words: End Stage Renal Disease. Periodontitis. Vitamin D receptor. Genetic polymorphisms

Introduction

Chronic kidney disease (CKD) is a progressive disorder that results from a profound hydroelectrolytical, metabolic and immunological imbalance, associated with a number of systemic complications [1]. In Brazil, the prevalence of patients enrolled in chronic programs of dialysis has increased more than twofold in the last 8 years. In 1994, there were 24,000 patients in dialysis programs. This number reached 59,153 patients in 2004, and the number of new patients increases by about 8% per year [2]. Recently, a number of nontraditional risk factors for mortality, such as chronic inflammation, oxidative stress and extra-osseous calcification, were identified in CKD patients, and the presence of these risk factors was associated with poor outcome in this population [3]. Chronic infections appear to be important causes of persistent systemic inflammation, and patients with CKD are more prone to infections than the general population [4]. Infectious diseases, such as periodontitis (PD), have also an impact in the morbidity of these patients [5]. Thus, metabolic, immunological and infectious complications have begun to form a great challenge in the clinical handling of this population.

PD, also known as periodontal disease, is an infectious disease of the oral cavity initiated by Gram-negative bacteria, characterized by inflammatory cell accumulation in the periodontal tissues [5]. According to the American Academy of Periodontology [6], 50% of adults have at least a moderate type of PD. In Brazil, 50% of the population between 35 and 44 years present with some form of PD, according to the Brazil Oral Health Project [7], PD has been considered a CKD complication [8], and its prevalence and severity are suggested to be increased in this population [8,9].

Vitamin D is a steroid hormone, metabolized in the liver and in the kidney, resulting in the vitamin D active form $1,25(OH)_2D_3$. This active form interacts with its nuclear receptor (VDR) in the intestine, bones, and kidneys to regulate a wide variety of biological processes including bone metabolism, modulation of the immune response and regulation of cell proliferation and differentiation [10]. Vitamin D is responsible for both positive and negative control of certain genes at the level of transcription, via interaction with VDR [11].

The human VDR is a product of a single gene, which is located on chromosome 12 at 12q13-14 [12]. The gene is comprised of 11 exons that, together with intervening introns, span approximately 75 kb [13]. A genome-wide observation expects over 100 polymorphisms in the VDR region [14]. Polymorphisms refer to the existence of 2 or more alleles at a given locus, and if such alleles occur at a frequency of more than 1% in a population, the locus is said to be polymorphic. Single-nucleotide polymorphisms (SNPs) are the most common form of DNA variation in the human genome. Near the 3'

untranslated region (UTR) there are 2 SNPs identified by the presence of restriction sites for *Bsm*I [15] and *Taq*I [16] restriction enzymes. Allelic variations in this region could be responsible for differences in translational efficiency or in messenger RNA stability, resulting in changes in cellular expression of VDR [17]. Such polymorphisms have been associated with several pathological conditions, such as breast cancer [18], bone mass, bone turnover, bone mineral loss [19], osteoarthritis [20], and diseases in which the bone loss is a cardinal signal, such as osteoporosis [21] and PD [22].

Complex diseases, such as CKD [23] and PD [24], have a genetic basis that combines the effects of the interaction of sequence variation of multiple genes with the environment [25]. Vitamin D has an impact in both CKD and PD by the fact that modulates bone metabolism and immune function which, in turn, are basic mechanisms that must be balanced to avoid damage. Our hypothesis is that polymorphisms in vitamin D receptor gene are associated with end-stage renal disease (ESRD) and PD. Thus, the aim of the present work was to investigate the association between polymorphisms in the VDR gene and CKD and PD.

Methods

Study population

A convenient sample of 222 unrelated patients, of both sexes, mean age 44.9 years (range 23-77) 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 4 groups:

Group 1: 59 individuals without CKD (glomerular filtration rate>90 mL/min estimated according to the Modification of Diet in Renal Disease formula [26]) and without PD;

Group 2: 50 patients without CKD and with PD [clinical attachment loss (CAL)>5 mm, in at least 3 teeth, in at least 2 quadrants];

Group 3: 50 patients with ESRD, in hemodialysis and without PD;

Group 4: 63 patients with ESRD, in hemodialysis and with PD.

Exclusion criteria were following: 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 acute necrotizing ulcerative gingivitis and PD.

Table 2 shows the general clinical aspects of ESRD patients.

Serum markers

C-reactive protein (CRP) was the marker of choice to measure systemic inflammation in all patients by means of nephelometry (method sensitivity 0.08 mg/L).

Laboratory measurement of several serum components was obtained according to the routine of the dialysis clinics for ESRD patients (table 2).

Clinical parameters of PD

Diagnosis of PD was made on the basis of clinical parameters, such as probing pocket depth and assessment CAL. Measurements 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 [27]. The following parameters were recorded: the gingival index [28]; the plaque index [29], the calculus index [30], and mobility (absent or present). The periodontal statuses of all subjects are shown in table 3.

DNA collection and Purification

Cells were obtained through a mouthwash with 3% glucose solution and scraping of the oral mucosa with a sterile spatula [31]. DNA was extracted from epithelial buccal cells with ammonium acetate 10 M and EDTA 1 mM [32].

Analysis of VDR Polymorphisms

VDR TaqI Polymorphism

The following primer pair was used for PCR amplification of genomic DNA samples: (F -5' CAG AGC ATG GAC AGG GAG CAA G 3' and R -5' GGA TGT ACG TCT GCA GTG TG 3'). Reaction conditions and cycling parameters were as follows: 2 µL of the genomic DNA were used for PCR amplification in a reaction mixture containing 45 µL PCR Supermix (Invitrogen Life Technologies, Carlsbad, Calif., USA), and 0.5 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. The restriction fragment length polymorphism (RFLP) technique was performed in a final reaction volume of 20 µL, using 2 units *Taq*I (T↓CGA) (Invitrogen Life Technologies), and 10 µL aliquot of PCR products, digested at 65ºC overnight. The

digested products were separated in 1.7% agarose gel electrophoresis visualized by ethidium bromide UVB illumination. The genotypes were determined by comparing the RFLP band patterns with a 1-kb-plus DNA ladder (Invitrogen Life Technologies). The RFLP is formed by a single-base transition (T/C) at codon 352 in exon 9 of the VDR gene that creates a *Taq*I restriction site. The alleles which result from the cleavage of *Taq*I are designated “C” (*Taq*I site present, with 2 fragments: 293 and 47 bp) or “T” (*Taq*I site absent, with a fragment: 340 bp).

*VDR Bsm*I *Polymorphism*

Specific oligonucleotide primers (F -5’CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA 3’ and R -5’AAC CAG CGG GAA GAG GTC AAG GG 3’) were used to amplify the fragment of the VDR gene including the *Bsm*I restriction site in intron 8. PCR was performed using denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 1 min, 63ºC for 1 min, 72ºC for 1 min and a final extension at 72ºC for 7 min. PCR products were digested with 2U *Bsm*I (New England Biolabs, Inc., Beverly, Mass., USA) (GAATGCN↓) at 65ºC ON. The resulting fragments were separated after electrophoresis on a 1.7% agarose gel and visualized by ethidium bromide UVB illumination. The RFLP is formed by a single-base transition (G/A) in intron 8 of the VDR gene that creates a *Bsm*I restriction site. The alleles which result from the cleavage of *Bsm*I are designated “A” (*Bsm*I site present, with 2 fragments: 646 and 177 bp) or “G” (*Bsm*I site absent, with a fragment of 823 bp).

Statistical Analysis

The significance of the differences in observed frequencies of each polymorphism between the groups was assessed by a standard χ^2, and differences were considered significant when $p < 0.05$. Comparisons between the 2 groups for nominal variables in tables 2x2 were made using Fisher’s exact test. Student’s t test was used to compare means from 2 groups. For the nonparametric variables, the Mann-Whitney U test was used to assess differences between groups. Continuous variables were expressed as means ± SD. Comparisons of continuous variables were performed using 1-way ANOVA. When ANOVA revealed differences, the Tukey (when variance was similar) and Games-Howell tests (when variance was not similar) were used. The Kruskal-Wallis test was used for nonparametric multiple comparisons for independent variables. The logistic-regression model was used to estimate the predictive factors which might influence the development of PD in ESRD patients. The physical proximity of the *Taq*I and *Bsm*I polymorphisms justifies the simultaneous analysis as haplotypes. The program package Arlequin 3.0 was used to calculate

haplotype frequencies, gene heterozygosity, Hardy-Weinberg expectations and linkage disequilibrium. The risk associated with genotypes, alleles and haplotypes was calculated as the odds ratio (OR) with 95% CI. Statistical analysis was performed using the statistical software BioEstat 2.0 for Windows, SPSS 10.0 for Windows (SPSS Inc, Chicago, IL., USA), and the program package Arlequin 3.0.

Results

Serum Markers

Increased values of the CRP were found in group 3 (with ESRD, without PD; 7.6±1.7) compared to group 1 (neither diseases; 2.9±1.4) (p=0.00). The highest levels of this inflammation marker were observed in group 4 (with ESRD and PD; 13.3±1.1).

Phosphorus levels were statistically different between ESRD groups with and without PD (p=0.00).

Clinical Findings in ESRD Patients

In ESRD patients, a weak relationship between PD and diabetes (p=0.07) and a significant association between PD and hypertension (p=0.01) was found. The logistic-regression model showed that age did influence the PD development in ESRD patients (p<0.01).

Genotyping and Disease Status

The frequencies of VDR *Taq*I and *Bsm*I genotypes/alleles/haplotypes can be observed in table 4. No significant differences between the 4 groups for the *Taq*I polymorphism were observed in relation to genotypes (p=0.98) or alleles (p=0.86). However, when the *Bsm*I polymorphism was analyzed, differences in genotypes (p=0.04) and alleles (p=0.01) were found. When combining the groups 1 and 2 (without CKD) versus 3 and 4 (with ESRD) for *Bsm*I polymorphism, statistically significant difference for genotype GG versus GA+AA (OR 2.5 95% CI 1.4-4.6, p=0.00) was observed. Regarding alleles A x G significant difference was observed when grouping 1/2 x 3/4 (OR 1.5 95% CI 1.0-2.3, p=0.02). In relation to haplotypes, grouping 1/2 x 3/4 (TG+CG vs. TA+CA), differences were also found (OR 1.5 95% CI 1.0–2.3, p=0.02). These results point to a protective effect for allele G against ESRD. No influence of the 2 study polymorphisms was found for susceptibility to or protection against PD.

Genotyping and ESRD *Clinical Parameters*

Certain alleles and genotypes were associated with clinical aspects of ESRD. Allele C for VDR *Taq*I polymorphism was shown to have a protective effect against

diabetes (p=0.00) and hepatitis (p=0.06), and to predispose to hypertension (p=0.01) in ESRD patients. Allele T for the VDR *Taq*I polymorphism (p=0.01) and genotype AA for the *Bsm*I polymorphism (p=0.05) were related with higher levels of serum creatinina.

Genotyping and PD Clinical Parameters

Regarding the clinical periodontal status, only the gingival index showed any significant association with *Taq*I T allele (p=0.04).

Discussion

The hormonally active form of vitamin D is involved in many biological functions throughout the body, such as regulation of calcium and phosphate homeostasis, bone remodeling, immune function, and gene expression [10]. Bone is one of the classical target organs for vitamin D action. Vitamin D has an essential role in bone development and mineralization [33]. Vitamin D facilitates these functions by controlling the availability of calcium and phosphate and by regulating the level of hormones such as parathyroid hormone (PTH) [34]. Vitamin D is also involved in the synthesis of bone matrix proteins such as type I collagen, alkaline phosphatase, osteocalcin and osteopontin [35,36]. Moreover, there is considerable scientific evidence that vitamin D has a variety of effects on immune system function that may enhance innate immunity and inhibit the development of autoimmunity [37]. Vitamin D inhibits lymphocyte proliferation and stimulates monocyte differentiation [12], and increases secretion of cytokines, such as interleukin (IL) -2, interferon-γ and IL-12 [38].

The vitamin D receptor is a ligand-activated transcriptional factor that mediates the effects of active vitamin D [11] in a wide variety of tissues and cells, including heart, stomach, pancreas, brain, skin, gonads [39,40], bone [41] and most cells of the immune system, including T cells, dendritic cells and macrophages [42]. The vitamin D receptor is also present in aortic endothelial [43] and vascular-smooth-muscle [44] cells. In the afferent glomerular arterioles of the kidney, vitamin D is a negative endocrine regulator of renin [45]. Renin is a rate-limiting component of the regulatory cascade that plays an essential role in the control of blood pressure [46], with potential effect in the cardiovascular system. Thus, it is not all surprising that vitamin D has a range of biologic effects that are noncalcemic in nature.

CKD is a progressive and irreversible decline in the total number of functioning nephrons, resulting in reduced glomerular filtration rate. Secondary hyperparathyroidism, disturbance of mineral-bone metabolism, and vascular calcification (which can lead to cardiovascular disease) represent the most common

complications leading to increased patient morbidity and mortality [47,48]. Besides, CKD patients are characterized by a severe impairment of immune function [49]. Regarding complications of an infectious nature, close attention has been given to PD in CKD patients [1]. Patients with CKD are at high risk of developing oral-health complications, such as increased prevalence of calculus [50] and PD [51] compared with the general population. Calculus and PD are in turn potential causes of sustained systemic inflammation in CKD patients [52]. Periodontal evaluations are not normally performed as part of a medical assessment in CKD patients. In support of this statement, destructive PDs have been reported to be more prevalent and severe in hemodialysis populations [53]. Similarly to CKD patients, individuals with PD show an impairment of the immune function and signs of alveolar bone loss resulting from an exacerbated inflammatory process [54].

In this study, patients with ESRD showed significantly higher levels of CRP, as shown, by other authors [55], which were further increased in ESRD patients with PD. Hepatic CRP synthesis is upregulated by inflammation and its level is a precise and objective index of inflammatory activity, reflecting the generation of proinflammatory cytokines that have proven to be a strong predictor of cardiovascular disease and outcome in dialysis patients [56]. Phosphorus was also significantly higher in ESRD patients with PD. Hyperphosphatemia stimulates PTH production which leads to bone resorption [57], being a significant cardiovascular risk factor in patients with CKD as a trigger for the extra-osseous calcification process [58]. From these findings, the presence of PD could represent an additional potential risk factor for mortality, more than morbidity, in ESRD patients.

Associations between PD and diabetes and between PD and hypertension were identified in the study in ESRD patients. Associations between PD and diabetes have also been found in non-CKD populations, maybe by the fact that the inflammatory process present in PD leads to increased levels of cytokines such as tumor necrosis factor α, which is known to foster insulin resistance [59]. Regarding hypertension, infectious processes involved in PD stimulate both local and systemic inflammatory responses in the host, and inflammatory mediators have the inherent potential to promote vascular changes that raise blood pressure [60].

Recent studies have indicated that there exist a number of polymorphisms in the VDR gene, but the influence of these polymorphisms on VDR protein function is largely unknown [38]. The role of polymorphic alleles is often shown by functional studies. The 3' UTR is known to be involved in the regulation of gene expression, especially through modification of mRNA stability [61]. Alleles of VDR *Taq*I and *Bsm*I polymorphisms seem to be in linkage disequilibrium (LD) with 3' regulatory region

containing the UTR [16]. Strong LD was also observed between the *Bsm*I polymorphism and poly(A) variable number of tandem repeats in the 3' UTR [62]. Polymorphisms *Taq*I and *Bsm*I in the VDR gene have been associated with prevalence and aggressiveness of prostate [63] and breast [18] cancer, aspects of bone mass [15], turnover and homeostasis [64], osteoporosis [21] and PD [22].

In this study, considering the 2 SNPs independently, the genotype distributions were consistent with the assumption of Hardy-Weinberg equilibrium.

The *Bsm*I polymorphism allele G played a role in protecting patients against end-stage renal disease development. This was maybe due to the fact that patients carrying this allele could have less cardiovascular complications leading to death. Some *in vitro* studies have shown a better response for haplotypes containing allele G to augment levels of mRNA expression in several cell types [16,17]. Other *in vivo* studies have correlated allele G with the serum concentration augmentation of bone formation markers [15,16]. Allele G was also observed to be involved in increased bone mineral density in response to treatment [65] (see Uitterlinden et al., 2004 [38] for details). Besides, the GG genotype seemed to slow the progression of secondary hyperparathyroidism [66]. Also, patients with CKD presenting severe secondary hyperparathyroidism showed lower serum PTH levels with a GG genotype [67], and individuals homozygous for AA presented higher levels of PTH and reduced bone density [68]. The reduced PTH levels may be related to lower extra-osseous calcification, preventing CKD patients from developing cardiovascular diseases, which are the major complications leading to poor outcomes and higher mortality.

Furthermore, individuals with genotype AA for *Bsm*I polymorphism (p=0.05) showed higher levels of serum creatinina, which is a classical serum marker for the presence of CKD. This finding could be an additional indication for allele G having a protective effect against CKD. In spite of evidence, some additional functional studies are needed to corroborate these results. In this segment, the authors are investigating VDR gene expression in the same group of patients by real-time PCR according to genotypes and haplotypes. No association was found between *Taq*I polymorphism and ESRD. However, allele C for the *Taq*I polymorphism was associated with protection against hepatitis and diabetes, and with susceptibility to hypertension in ESRD individuals. This allele was reported to be in LD with *Bsm*I allele G [69].

With regard to PD, no differences in the allele or genotype distribution of *Taq*I or *Bsm*I polymorphisms were observed. However, allele T for *Taq*I polymorphism was associated with the gingival index, which is an indicator of tissue inflammation. Allele T has been shown to increase VDR expression and augment VDR mRNA stability *in*

vitro, and was reported to be in LD with allele A for *Bsm*I polymorphism [69]. Alleles of these VDR polymorphisms were associated with PD in previous studies [22,70].

Both CKD and PD are serious public-health concerns all over the world, but the latter has been especially neglected in the CKD population [9,50,63]. Understanding the association between these 2 common diseases and the mechanisms underlying this association will aid health professionals to provide improved means to prevent, diagnose, and handle CKD complications in this particularly susceptible population. The mouth is a potential focus of infection, which may interfere with the progression of systemic diseases by eliciting the host inflammatory response [71]. Handling patients as a whole is of particular importance in certain complex disease systems.

Conclusion

In summary, the association between *Taq*I and *Bsm*I polymorphisms in the VDR gene and ESRD and PD was investigated. Allele G for *Bsm*I polymorphism was associated with protection against ESRD in a Brazilian population. Other studies considering a higher number of individuals matched by sex and age should be conducted to replicate these findings.

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Table 1. Baseline characteristics of the study population.

	Group 1 (n=59)	Group 2 (n=50)	Group 3 (n=50)	Group 4 (n=63)	*p* value
Ethnic Group n (%)					
Caucasoid	46 (77.9)	38 (76)	35 (70)	44 (69.8)	
Afro-American	9 (15.3)	11 (22)	13 (26)	15 (23.8)	0.67 *
Mulatto	4 (6.8)	1 (2)	2 (4)	4 (6.4)	
Age years (range) †	37.9±9.6 (20-70)	40.8±9.4 (20-61)	45.2±12.9 (23-74)	54.2±12.1 (26-77)	0.00 **
Gender n (%)					
Female	43 (72.8)	33 (66)	13 (34)	23 (36.5)	0.00 *
Male	16 (27.2)	17 (34)	33 (66)	40 (63.5)	

†Mean±Standart Deviation; * Chi-square; **ANOVA.
The difference observed among groups in the mean age and gender is due to most ESRD patients being older and male. However, Binary Logistic Regression showed no influence of sex and age on the genotype distribution.
Group 1: healthy patients. Group 2: without CKD and with PD. Group 3: with ESRD and without PD. Group 4: presenting ESRD and PD.

Table 2. General clinical aspects of ESRD patients.

	Without PD [a] (n=50)	With PD (n=63)	*p* value
Age years *(range) †*	45.2±12.9 (23-74)	54.2±12.1 (26-77)	
Main cause of CKD [b] n (%)			
Chronic glomerulonephritis	19 (38)	20 (31.7)	
Hypertensive nephropathy	14 (28)	10 (15.9)	
Diabetic nephropathy	7 (14)	14 (22.2)	
Other/Unknown	10 (20)	19 (30.2)	
Duration of HD [c] ***treatment (months)*** [†]	47.8±48.0	47.2±43.3	
General medical condition n (%)			
Diabetes	7 (14)	17 (26.9)	0.07 *
Hepatitis	11 (22)	17 (26.9)	
CVD [d]	10 (20)	16 (25.4)	
Hypertension	33 (66)	54 (85.7)	0.01 *
Current medication n (%)			
Antihypertensives	35 (70)	49 (77.7)	
Diuretics	10 (2)	23 (36.5)	
Calcium carbonate	34 (68)	46 (73)	
Vitamin D (calcitriol)	9 (18)	7 (11.1)	
Antiplatelet agents	3 (6)	5 (7.9)	
Others	41 (82)	51 (80.9)	
Habits n (%)			
Smoking	11 (22)	16 (25.3)	
Laboratory measurements [†]			
CRP [e] (mg/L)	7.6±10.2	13.3±18.2	0.00 **
Serum creatinine (mg/L)	10.2±2.8	9.5±2.7	
Normal protein catabolic rate	0.9±0.2	0.9±0.2	
Kt/V [f]	1.3±0.2	1.2±0.2	
Serum calcium (mg/dL)	8.9±0.7	9.1±0.7	
Serum phosphorus (mg/dL)	5.2±1.2	6.5±1.1	0.00 **
Serum calcium-phosphorus product (mg/dL)	49.4±1.8	51.9 ±1.6	
Serum potassium (mg/dL)	5.2±0.5	5.3±0.6	
PTHi [g]	458.9±556.6	453.4±496.0	
Serum alkaline phosphatase (U/L)	125.5±120.9	114.4±69.6	
Serum albumin (mg/dL)	3.7±0.3	3.6±0.3	
Ferritin (ng/mL)	697.1±289.4	715.7±314.9	
Leucocytes	6722.3±1372.1	6898.7±1694.9	
Hemoglobin	11.5±1.6	11.3±1.4	
Hematocrit (%)	34.8±4.2	34.2±4.7	

[a]Periodontal disease; [b]Chronic kidney disease; [c]Hemodialysis; [d]Cardiovascular disease; [e]C-reactive protein; [f]Marker of dialytic adequacy; [g]Serum intact parathormone. [†]Mean±Standart Deviation; *Fisher test; ** t student test.

Table 3. Periodontal status of study population.

Periodontal Status	Group 1 (n=59)	Group 2 (n=50)	Group 3 (n=50)	Group 4 (n=63)	*p* value
Gingival Index †	0.2±0.1	1.5±0.1	0.4±0.1	1.6±0.1	0.00 *
Plaque Index †	0.3±0.4	1.3±1.0	0.6±0.8	0.9±0.9	0.00 *
Calculus Index †	0.2±0.2	1.0±0.9	0.3±0.5	0.7±0.8	0.00 *
PPD [a] (mm) †	1.5±2.4	4.6±1.0	2.1±2.0	3.5±1.1	0.00 *
CAL [b] (mm) †	2.2±2.6	6.1±1.0	2.6±2.7	5.3±1.3	0.00 *
Mobility (presence /absence)	0/59	21/29	0/50	31/32	0.00 **

[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 ESRD and without PD. Group 4: presenting ESRD and PD.

Table 4. Allelic and genotypic distribution of VDR gene *Taq*I and *Bsm*I polymorphisms.

Polymorphisms n (%)	Group 1 (n=59)	Group 2 (n=50)	Group 3 (n=50)	Group 4 (n=63)	Chi-square *p* value
*Taq*I Genotypes					
T T	23 (38.9)	20 (40.0)	23 (46.0)	24 (38.9)	χ^2=1.15
T C	29 (49.1)	24 (48.0)	23 (46.0)	32 (50.0)	*p*=0.98
C C	7 (12.0)	6 (12.0)	4 (8.0)	7 (11.1)	
Alleles					
T	75 (63.5)	64 (64.0)	69 (69.0)	80 (63.4)	χ^2=0.76
C	43 (36.5)	36 (36.0)	31 (31.0)	46 (36.6)	*p*=0.86
*Bsm*I Genotypes [a]					
G G	22 (37.3)	21(42.0)	11 (22.0)	12 (19.1)	χ^2=12.76
G A	22 (37.3)	22 (44.0)	26 (52.0)	37 (58.7)	*p*=0.04
A A	15 (25.4)	7 (14.0)	13 (26.0)	14 (22.2)	
Alleles [b]					
G	66 (55.9)	64 (64.0)	48 (48.0)	61 (48.4)	χ^2=10.19
A	52 (44.1)	36 (36.0)	52 (52.0)	65 (51.6)	*p*=0.01
Haplotypes [c]	Group 1 + Group 2 (n=109)		Group 3 + Group 4 (n=113)		
(T G + CG)	130 (59.6)		109 (50.5)		χ^2=3.68
(T A + CA)	88 (40.4)		107 (49.5)		*p*=0.05

[a]Groups 1/2 versus 3/4 (GG) x (GA+AA): [OR=2.5; 95%; CI=1.4-4.6 (*p*=0.00)]; [b]Groups 1/2 versus 3/4 (G x A): [OR=1.5; 95%; CI=1.0-2.3 (*p*=0.02)]; [c]Groups 1/2 versus 3/4 (TG+CG) x (TA+CA): [OR=1.5; 95% ; CI=1.0-2.3 (*p*=0.02)].

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

Conclusões

CONCLUSÃO

Foi observada evidência de associação entre o alelo G do polimorfismo *Bsm*I do gene do VDR e a proteção contra a DRC. Nenhuma associação foi observada entre os polimorfismos estudados e a suscetibilidade ou a proteção contra a DP. Foi concluído que o alelo G do polimorfismo *Bsm*I do gene do VDR parece apresentar um efeito protetor na progressão da DRC.

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Anexos

Artigo 2

Aceito para publicação na Revista Médica do Chile (*qualis* C int. em jul. 2007)

Oral health status of Brazilian patients with chronic kidney disease

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Running title: Oral health status in Brazilian renal patients

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Background. Poor oral health status has an impact on renal patients' morbidity. This study aimed to describe retrospectively the oral health status of a chronic kidney disease (CKD) Brazilian population. ***Aims:*** The aim of this study was to describe the oral health status of a CKD Brazilian population. ***Materials and methods****:* Two hundred eighty-six records: 4.5% in the predialysis (Pre), 8.4% on peritoneal dialysis (PD), 55% on hemodialysis (HD), and 32.1% transplanted (Tx) were analyzed. General oral health, presence of dental calculus, and halitosis were investigated based on analysis of patient records. The number of decayed, missed and filled teeth was analyzed by means of DMF-T index. ***Results****:* The study population was composed of 152 men (53%) and 134 women (47%), and the mean age was 42±13 years. The general oral health status was considered defective to most patients (83%). The great majority of patients presented dental calculus (86.7%). Halitosis was reported by 55% of patients, and transplant patients (Tx) reported less halitosis (40.2%) when compared to groups Pre (69.2%), HD (60.7%), and PD (69.6%) (p=0.004). The DMF-T for the whole population was 20.6. The defective oral health correlated to dental caries (p=0.005). ***Conclusions:*** Evaluated parameters suggest poor oral health status in Brazilian CKD patients in different modalities of treatment. Supportive dental programs must be established in order to minimize pathogen influence, which may predispose to systemic complications.

(***Key words:*** *Chronic kidney disease; Oral health; Calculus; Halitosis; Caries*)

Chronic kidney disease (CKD) is characterized by a number of systemic complications that result from a profound hidroelectrolytic, metabolic, and immunological imbalance. Despite the improvements in patient care and renal replacement therapy, the impact of CKD on patient's morbidity and mortality is extremely high[1]. Recently, a number of non-traditional risk factors for mortality such as chronic inflammation, oxidative stress and extra-osseous calcification were identified in CKD patients, and the presence of those risk factors was associated with poor outcomes in this population[2].

Chronic infections appear to be important causes of persistent systemic inflammation and generation of oxidative stress, and patients with CKD are more prone to infections than the general population. Signs of sustained chronic inflammation are present in most CKD patients, but causative mechanisms are yet to be clarified. In addition, abnormalities in the calcium and phosphorus metabolism are highly prevalent in the CKD population, and this mineral imbalance is associated with increased mortality in this group of patients. Strategies to improve CKD patient's outcome will most likely need to focus on the reduction of those risk factors[3].

Patients presenting CKD are at high risk to develop oral health complications, such as narrowing of pulp chamber[4], enamel abnormalities[5], xerostomia[6], premature tooth loss[7], increased prevalence of calculus[8], and periodontal disease[9], when compared to the general population. Since CKD patients present medical, psychological, and socio-economical characteristics that may predispose to odontological problems, oral health in dialysis and transplant patients has been proposed to be poor, with a potential impact on patient's morbidity, mortality and quality of life[6,10].

Uremic halitus is a well known clinical characteristic of CKD patients, but comparison between the prevalence of this complication in different forms of renal replacement therapy has not been described until the present. In addition, poor oral hygiene and development of dental calculus are risk factors for periodontal disease, which in turn is a potential cause of sustained systemic inflammation in patients with CKD[25]. Only a few studies performed in a limited number of patients reported oral health status in CKD patients[7,8,11,14], and no reports of oral health status performed in a clinic specialized in oral care of CKD patients are available. Moreover, a comparison of oral health status between pre-dialysis, hemodialysis, peritoneal dialysis, and transplant patients has not been described until the present. Therefore, the aim of this study was to report some parameters of oral health status in a population of patients undergoing different modalities of CKD treatment.

MATERIAL AND METHODS

During the years of 2001-2005, 1,829 CKD patients were treated for their CKD in the predialysis (Pre; n=456), hemodialysis (HD; n=799), peritoneal dialysis (PD; n=179), and transplant (Tx; n=395) clinics. Out of these, about 300 patients attended the dental clinic of the Pro-Renal Foundation, where patients were evaluated and followed up for dental diseases with a focus on CKD. The dental clinic fits an area of 41 m^2, and counts with three dental cabinets, which are used by two dentists, assisted by dental students from local universities.

Two hundred eighty-six (286) patient records were carefully reviewed by dentists, which represent 16 % of the pool of patients treated at the clinics during this period. Out of the patients, 13 (4.5 %) were Pre, 158 (55%) were HD, 23 (8.4%) were PD and 92 (32.1%) were Tx patients. The study was approved by the Ethical Committee in Research at PUCPR (approved under protocol 264/10184). The data extracted from the records were: reported main cause of CKD, general medical condition, current medications, and smoking. With regards to the oral status, the following aspects were considered: general oral health condition (good or defective), presence of dental calculus, and halitosis. The number of decayed, missed and filled teeth was computed by means of an index, recognized by DMF-T (Decayed, Missed and Filled Teeth). DMF-T is a numerical representation that indicates the prevalence of tooth decay individually or in a certain population. It is an evaluation method, which is accepted by the international community as an oral health indicator. It is calculated by adding the number of decayed (D), missed (M) and filled (F) teeth (T) which is recommended by the World Health Organization (WHO)[15,16]. The criteria for classifying calculus were according to WHO recommendations. Calculus was evaluated by observing or noticing its presence recovering the dental surface.

Values were presented as mean ± standard deviation. Paired Student's t-test was used to investigate the oral health status in relation to caries mean. Analysis of variance (ANOVA) was used to compare differences between different groups. Prevalence of each condition was compared using the chi-square analysis incorporating Yates' correction. Pearson Correlation was used to evaluate association between age and DMF-T. Calculations were performed using the JMP package for Windows (version 7.0, USA) and a *p* value of less than 0.05 was judged to be significant.

RESULTS

The study population was composed of 152 men (53%) and 134 women (47%), and the mean age was 42±13 years. Table 1 shows the baseline clinical parameters of CKD patients according to the modalities of treatment.

The general oral health status was considered defective to most (233/286) patients (83%), regardless the modality of treatment (Fig. 1). The great majority of patients (248/286) presented dental calculus (86.7%) for all modalities of treatment (Fig. 1). Halitosis was reported by 158/286 (55%) patients. Transplant patients (Tx) reported less halitosis (40.2%) (p=0.004) when compared to groups Pre (69.2%), HD (60.7%), and PD (69.6%) (Fig. 1).

The DMF-T for the whole population was 20.6 The DMF-T for all forms of treatment is shown in figure 2. There was a significant correlation between age and DMF-T (r=0.21; p<0.0001). Patients with defective oral health condition presented a higher number of dental caries (6.7±0.3) when compared to patients with good oral health condition (4.7±0.6; p=0.005). The average of tooth decay was 5.4±4.4, and 9.7±8.7 teeth were missing.

DISCUSSION AND CONCLUSION

The rapidly increasing number of patients with CKD combined with the unacceptably high morbidity and mortality in patients receiving dialysis or a renal transplant points to an urgent understanding of the mechanisms responsible for the development of complications of CKD. From this point of view, the evaluation and promotion of oral health appears to be an important component of CKD care. It is necessary for the dental practitioner to thoroughly understand the special needs that arise from these patients[13]. This study was a retrospective and cross-sectional analysis that aimed to evaluate the oral health status in a large group of Brazilian patients followed in a clinic specialized in dental aspects of CKD. The main findings of this study were that CKD patients present defective general oral health status and a high prevalence of dental calculus, factors that may predispose these patients to oral diseases.

Patients with CKD present several relevant oral health problems, including uremic halitus, dry mouth, and taste and salivary alterations[12,18,19]. It has also been observed increased prevalence of caries, gingivitis, dental mobility, tooth loss, and dental calculus in this population[7]. General oral health condition was considered

defective for most patients of the study population and correlated to dental caries. Indeed, previous studies have suggested that oral hygiene of hemodialysis patients is worse than that for the general population[8,11,13,14]. Bad hygiene may be explained on the basis of the chronic nature of the illness. Patients are concerned about their renal disease and tend to neglect preventive measures related to other health aspects. Renal dysfunction has been also associated with psychological implications. These patients experience the stress of complying with frustrating dietary restrictions that have been found to contribute to anxiety reactions or depression[20,22].

The great majority of patients presented dental calculus. Indeed, in a study that analyzed the prevalence of calculus in a population from the same region and of similar age[23] showed that the prevalence of calculus was 40%, more than half the prevalence described in CKD patients. Dialysis patients may form calculus more rapidly than healthy individuals, possibly due to high salivary urea and phosphate levels[24]. Other important risk factors for the development of dental calculus are the ingestion of large quantities of calcium carbonate (used as a phosphate binder), hyperparathyroidism, and deficient hygiene[8,25,26]. The impact of the high prevalence of calculus on periodontal disease remains to be investigated in future studies.

Halitosis can also result from poor oral health. Other causes of halitosis and uremic fetor are xerostomia and the presence of urease-splitting oral organisms, which metabolize urea (present in high levels in these patients) and thus elaborate ammonia[6]. In our study, transplant patients reported less halitosis when compared to groups of patients with CKD before the transplant, possibly indicating that uremic toxin levels may be the main determinant of halitosis in the CKD population.

Dental caries is among the most common oral health problems all over the world. They occur in between 50 and 99% of people in most communities. The patients in the present study presented a DMF-T equal to 20.6, equivalent to that reported for the South population of Brazil (DMF-T = 20.6)[27]. Higher prevalence of caries has been reported in CKD patients[28]. However, a study[29] showed a low rate of caries in hemodialysis patients, what was thought to be related to a possible antibacterial effect of urea or increased calculus. Although the DMF-T in the CKD patients was identical to the general population of same sex and age, poor hygiene correlated to dental caries in the study population.

According to WHO, good health is a major resource for social, economic and personal development, and an important dimension of quality of life. Health promotion action aims at making these conditions favourable through advocacy for health[30]. Oral health could be explained as a status of complete normality of teeth and support structures in both morphological and functional ways and, also, of the perioral parts

and structures related to mastication and maxillofacial complex. Especially in compromised individuals, like CKD patients, oral environment might be assisted in order to promote not only oral but general health. Since one of the major complications in CKD patients is sepsis, and knowing that infectious diseases in the oral cavity may act as foci for systemic diseases or injury in other sites of the body, oral health must be achieved and maintained during all the CKD patient treatment period[8,31].

Evaluated parameters suggest defective oral health status in chronic kidney disease patients undergoing different modalities of treatment. Supportive dental programs must be established in order to minimize pathogens influence, which may predispose to systemic complications.

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LEGEND FOR THE FIGURES

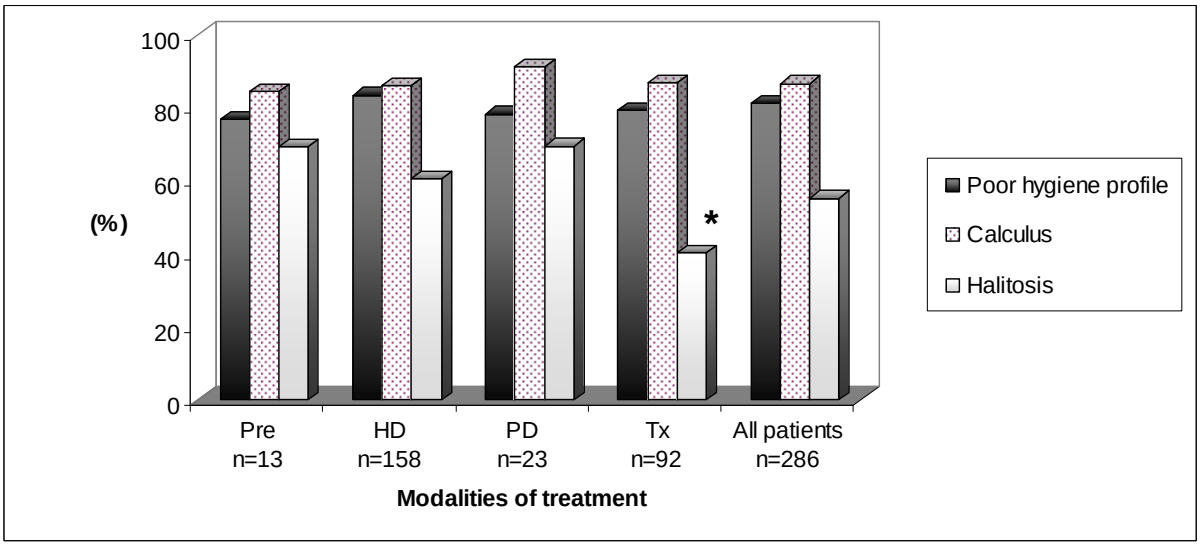
Figure 1 Prevalence of poor hygiene profile, halitosis and dental calculus in patients with chronic kidney disease.

Figure 2 DMF-T index for the CKD patients according to the modality of treatment.

Table 1: Main characteristics of the study population

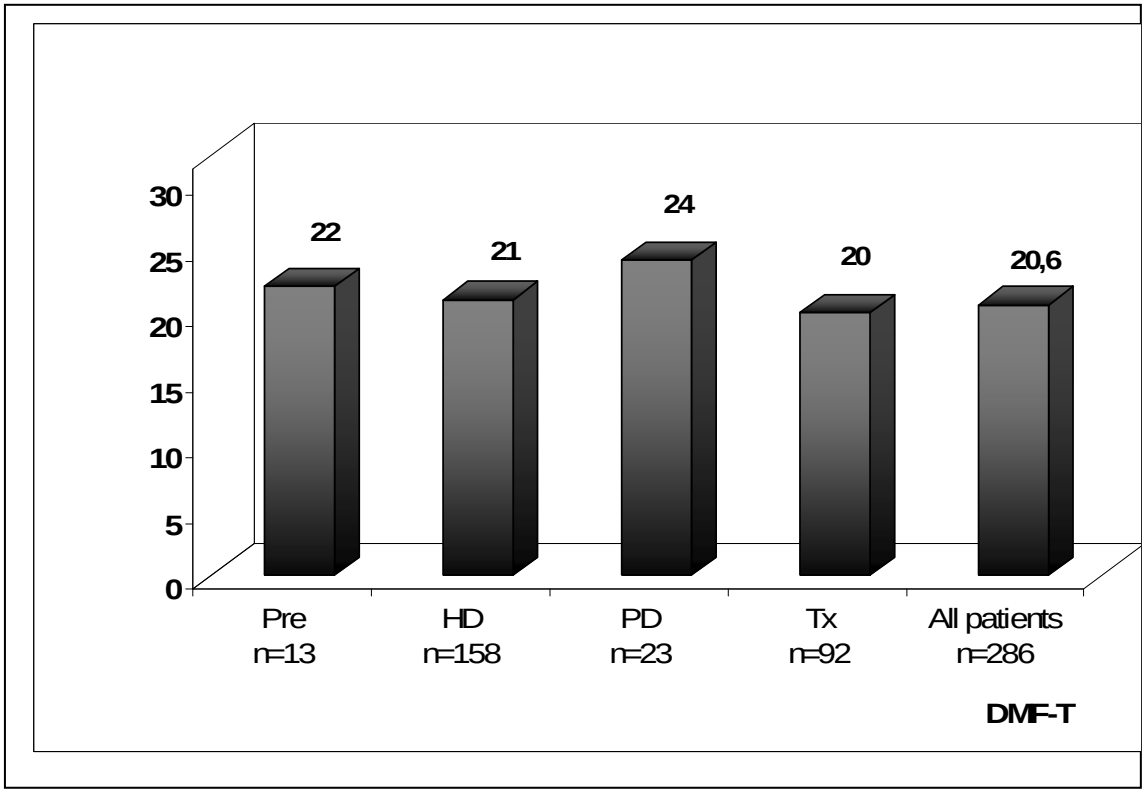
	n (%)
Gender	
Males	152 (53)
Females	134 (47)
Modalities of treatment	
Pre-dialysis (PRE)	13 (4)
Hemodialysis (HD)	158 (55)
Peritoneal dialysis (PD)	23 (8)
Transplant (Tx)	92 (33)
Main cause of CKD	
Chronic glomerulonephritis	62 (22)
Hypertensive nephropathy	53 (18)
Diabetic nephropathy	25 (8)
Other / Unknown	147 (51)
General medical condition	
Diabetes	56 (19)
Hepatitis	73 (26)
Current medication	
Antihypertensives	114 (40)
Diuretics	70 (24)
Calcium carbonate	163 (57)
Antiplatelet agents	35 (12)
Vitamins	119 (42)
Others	162 (57)
Smokers	57 (20)

Fig. 1



*Pre – predialysis; HD – hemodialysis; PD – peritoneal dialysis; Tx – transplant. *Prevalence of halitosis in transplant patients was significantly lower than the observed in HD, PD and Pre patients.*

Fig. 2



Pre – predialysis; HD – hemodialysis; PD – peritoneal dialysis; Tx – transplant; DMF-T – Decayed, Missing and Filled Teeth.