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Analysis of the association of an *MMP1* promoter polymorphism and transcript levels with chronic periodontitis and end-stage renal disease in a Brazilian population

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ABSTRACT

Chronic periodontitis (CP) and end-stage renal disease (ESRD) are complex inflammatory conditions. Higher levels of MMP-1 were found in fluids and gingival tissues from CP patients and in the blood and tissues from ESRD patients. *MMP1*-1607 (1G/2G) is a functional polymorphism, as it alters MMP-1 expression.

Objective: The aim of this study was to investigate the association of the *MMP1*-1607 (1G/2G) polymorphism with CP and ESRD and evaluate differences in transcript levels between the groups.

Design: A total of 254 individuals were divided into four groups: *Group 1*, without CP and without chronic kidney disease (CKD) ($n = 67$); *Group 2*, with CP and without CKD ($n = 60$); *Group 3*, without CP and with CKD stage 5 (ESRD) ($n = 52$), and *Group 4*, with CP and with ESRD ($n = 75$). The *MMP1*-1607 polymorphism was analysed by PCR-RFLP. *MMP1* gene transcripts from gingival tissues were analysed by real-time PCR.

Results: No association was found between the *MMP1*-1607 polymorphism and CP or ESRD. Increased levels of *MMP1* transcripts were observed in CP patients with or without ESRD. No differences were observed in the transcript levels according to the genotypes.

Conclusion: It was concluded that the *MMP1*-1607 polymorphism was not associated with either CP or ESRD. However, higher levels of *MMP1* gene transcripts were found at gingival sites of CP in patients both with and without ESRD.

1. Introduction

Matrix metalloproteinases (MMPs) represent a family of dependent metal ion endopeptidases, which are capable of degrading all extracellular matrix (ECM) components.[1] MMPs are classified by substrate specificity into collagenases, gelatinases, stromelysins, and membrane-bound types.[2] MMP expression is regulated by cytokines. Whereas T-helper type 1 (Th1) and the inflammatory mediators interleukin (IL)-1β, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ have been described as positive regulators of MMP expression, the

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reverse effect is exerted by the Th2-type cytokines, such as IL-4, IL-10 and IL-13.[3] The activation of MMPs is regulated by a group of endogenous proteins called tissue inhibitors of metalloproteinases (TIMPs) that are each able to inhibit nearly every member of the MMP family in a non-specific manner.[4] MMPs play an important role in physiological events during embryonic development, morphogenesis, angiogenesis, and tissue repair[5] and are overexpressed in several diseases, such as ovarian cancer,[6] atherosclerosis,[7] and osteoarthritis.[8] By their ability to degrade ECM, MMPs are at the crossroads of several disease progression and regression pathways.

Chronic periodontitis (CP) is a multifactorial disease in which the susceptibility, progression and outcome are dependent on multiple environmental and genetic factors.[9] CP is a highly prevalent infectious illness of the oral cavity that may affect at least 50% of adults, initiated by gram-negative bacteria and characterised by inflammatory cell accumulation in the periodontal tissues.[10] 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.[11] The inflammatory reaction is thought to trigger periodontal tissue destruction as a consequence of an imbalance in the expression of MMPs *versus* (*vs.*) TIMPs, which act to regulate extracellular matrix turnover of periodontal tissues, including alveolar bone.[12] The transcriptional levels and activity of MMPs are significantly higher in gingival tissues of individuals with CP than in healthy patients[13] and, as a consequence, destructive periodontal disease occurs in CP individuals.[10]

An emerging body of evidence suggests that oral inflammatory diseases, and particularly periodontal infections, may not be limited to the immediate oral environment but can have systemic effects.[14] Periodontitis has been considered as a complicator for several systemic diseases, such as chronic kidney disease (CKD),[15] and its prevalence and severity are thought to be increased within this disease population.[16] Destructive CP may be a significant source of inflammation in compromised patients when periodontal evaluations are not performed as part of a medical assessment.[17]

Chronic kidney disease is a progressive disorder associated with a number of systemic complications that is characterised by the destruction of the kidneys' functional units (nephrons) resulting from a profound hydroelectrolytic, metabolic and immunological imbalance.[18] CKD can result from a wide spectrum of diseases, such as diabetes, hypertension, glomerulonephritis, and autoimmune disorders.[19] Kidney disease is divided into five stages of increasing severity.[20] Independently of its aetiology, CKD can progress to an advanced stage, or renal disease stage 5, and designated as end-stage renal disease (ESRD) in which the signs and symptoms of uraemia (uraemic syndrome) predominate. Remodelling of the ECM is a key event in the progression and reversal of kidney disease. CKD results from a process in which there is disequilibrium between the increased synthesis of ECM components and decreased ECM degradation, primarily 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, primarily type-IV collagen.[21] In fact, BM damage is a major event in crescentic glomerulonephritis. Conversely, the excessive matrix accumulation observed in the fibrotic kidney results from a combination of overproduction and defective degradation of matrix components.[21] However, considering the multiplicity of their targets and the complexity of their regulation, MMP-mediated effects may be different and even opposite during the different phases of the evolution of nephropathies. As described for CP, altered cytokine production may result in the disturbance of MMP/TIMP balance.[22] In fact, excessive or inappropriate expression of MMPs has been associated with CKD complications, such as progressive renal injury, glomerular sclerosis,[23] interstitial kidney fibrosis,[24] and cardiovascular diseases.[25]

MMP-1 is a collagenase produced by fibroblasts, keratinocytes, endothelial cells, macrophages, osteoblasts and chondrocytes.[26] This enzyme 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.[27] MMP-1 is the major proteolytic enzyme that can cleave native interstitial collagen type I and III, which are the most abundant protein components of the ECM. Therefore, variance in MMP-1 transcription levels may be relevant to the progression of both CP and CKD. The *MMP1* gene is located in 11q22[28] and includes several functional polymorphisms located in the promoter region.[29] An insertion/deletion of a guanine at position -1607 of the human *MMP1* gene creates two different alleles: one with a single guanine (1G) and another with two guanines (2G).[30] It has been shown that the 1G/2G polymorphism is functional because the allele 2G significantly increases the transcriptional activity of MMP-1.[30] The presence of the allele 2G was observed to be associated with ovarian cancer,[31] renal carcinoma,[32] and CP.[33]

Although there are a few studies of ESRD and CP that investigate their association with the *MMP1*-1607 gene polymorphism, there is no study investigating the association of the *MMP1*-1607 gene polymorphism with CP in ESRD patients. Thus, the aim of the present work was to analyse the association between the *MMP1*-1607 polymorphism and *MMP1* transcript levels with CP and ESRD.

2. Materials and methods

2.1. *Study population*

A sample of 254 unrelated patients of both genders with a mean age of 44.6 years (range 20–77) was selected from the Dental Clinics of Pontifical Catholic University of Paraná (PUCPR) and from the Dental Clinics of the Pro-Renal Foundation over a period of two years between 2007 and 2009. All patients were from the southern region of Brazil. The baseline clinical parameters for the entire population are listed in Table 1. Although the study sample was primarily composed of Caucasians, the Brazilian white population is heterogeneous. A recent article suggested not to group Brazilians into ethnic groups based on colour, race and geographical origin because Brazilian individuals classified as white or black have significantly overlapping genotypes, probably due to miscegenation.[34] According to the Brazilian Government Census (2005), in southern Brazil, the racial