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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 patients both with and without ESRD.

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1. Introduction

Matrix metalloproteinases (MMPs) represent a family of dependent metal ion endopeptidases, which are capable of degrading all extracellular matrix (ECM) components. 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...
reverse effect is exerted by the Th2-type cytokines, such as IL-4, IL-10 and IL-13. 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. MMPs play an important role in physiological events during embryonic development, morphogenesis, angiogenesis, and tissue repair and are overexpressed in several diseases, such as ovarian cancer, atherosclerosis, and osteoarthritis. 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. 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. 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. 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. The transcriptional levels and activity of MMPs are significantly higher in gingival tissues of individuals with CP than in healthy patients and, as a consequence, destructive periodontal disease occurs in CP individuals.

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. Periodontitis has been considered as a complicator for several systemic diseases, such as chronic kidney disease (CKD), and its prevalence and severity are thought to be increased within this disease population. Destructive CP may be a significant source of inflammation in compromised patients when periodontal evaluations are not performed as part of a medical assessment. 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. CKD can result from a wide spectrum of diseases, such as diabetes, hypertension, glomerulonephritis, and autoimmune disorders. Kidney disease is divided into five stages of increasing severity. Independently of its aetiologies, 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 anaemia (uremic 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. 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. 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. In fact, excessive or inappropriate expression of MMPs has been associated with CKD complications, such as progressive renal injury, glomerular sclerosis, interstitial kidney fibrosis, and cardiovascular diseases.

MMP-1 is a collagenase produced by fibroblasts, keratinocytes, endothelial cells, macrophages, osteoblasts and chondrocytes. 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. 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 and includes several functional polymorphisms located in the promoter region. 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). It has been shown that the 1G/2G polymorphism is functional because the allele 2G significantly increases the transcriptional activity of MMP-1. The presence of the allele 2G was observed to be associated with ovarian cancer, renal carcinoma, and CP.

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. According to the Brazilian Government Census (2005), in southern Brazil, the racial
composition is 77.8% white, 2.2% black, 18.9% mixed-race, and 1.1% Japanese. Amongst the white population, there is a predominance of Italian, Spanish, and Portuguese heritage. All subjects completed personal, medical and dental history questionnaires that were within a protocol approved by an Institutional Review Board, and all participants signed consent forms 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)\(^{35}\) and without CP;
- **Group 2**: 60 patients without CKD and with CP [clinical attachment loss (CAL) ≥ 5 mm, in at least 3 teeth, in at least 2 quadrants];
- **Group 3**: 52 patients with CKD stage 5 (ESRD), in haemodialysis, and without CP; and
- **Group 4**: 75 patients with CKD stage 5 (ESRD), in haemodialysis, and with CP.

All CKD patients underwent dialysis sessions with polysulfone low-flux membranes, and the dialysate calcium concentration was 3.5 mg/L during the study. The dialysis time (3.5–5 h) and dose was delivered to achieve a Kt/V above 1.2. Haemoglobin levels were monitored to achieve the K/DOQI recommended parameters, and all patients were placed on epoetin alpha therapy. The target for ferritin was between 200 and 800 ng/mL, and intravenous iron saccharose was used when needed. Calcitriol and phosphorus binders were prescribed as needed to achieve the mineral metabolism targets recommended by the K/DOQI guidelines.

Patients were not included if they presented chronic use of anti-inflammatory drugs, AIDS, immunosuppressive chemotherapy, systemic active infection, current pregnancy or lactation, use of orthodontic appliances, or presence of necrotising ulcerative gingivitis or periodontitis. Smokers were included, but only 37/254 (14.5%) claimed smoking habits, and no statistically significant difference was observed between the groups with and without CP in patients with and without ESRD.

### 2.2. Clinical parameters for CP

Diagnosis of CP was made based on clinical parameters, such as probing pocket depth (PPD) and assessment of CAL. Measurements of PPD and CAL were recorded at 4 points around each tooth. Subjects with CAL > 5 mm, in at least 3 teeth and at least 2 quadrants, were considered affected.\(^{36}\) The following parameters were recorded: gingival index (GI),\(^{37}\) plaque index (PI),\(^{38}\) calculus index (CI),\(^{39}\) and mobility (absent or present). The periodontal status of all subjects is shown in Table 2.

### 2.3. Biochemical parameters of ESRD patients

The following blood cells and serum markers were measured according to the routine of the Dialysis Clinics for ESRD patients: C-reactive protein, creatinine, protein catabolic rate, calcium × phosphorus, Kt/V (a marker of dialysis adequacy), percentage of reduction urea, urea, potassium, haematoцит, alanine aminotransferase (ALT), albumin, iron, glucose, calcium, phosphorus, ferritin, transferrin, iron saturation index, haemoglobin, alkaline phosphatase, leukocytes, and aluminium. The general clinical aspects of ESRD patients either without (group 3) or with (group 4) CP can be found in Table 3.

### 2.4. Analysis of MMP1-1607 (1G/2G) polymorphism by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)

Cells were obtained through a mouthwash with 3% glucose solution and scraping of the oral mucosa with a sterile spatula.\(^{40}\) DNA was extracted from epithelial buccal cells with 8 M ammonium acetate and 1 mM EDTA.\(^{41}\)

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**Table 1 – Baseline characteristics of the study population.**

<table>
<thead>
<tr>
<th>Ethnic Group, n (%)</th>
<th>Group 1*(^{1}(n = 67))</th>
<th>Group 2(^{1}(n = 60))</th>
<th>Group 3(^{1}(n = 52))</th>
<th>Group 4(^{5}(n = 75))</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>53 (79.3)</td>
<td>46 (76.7)</td>
<td>38 (73.1)</td>
<td>52 (69.3)</td>
<td>0.646*</td>
</tr>
<tr>
<td>Mulatto</td>
<td>10 (14.9)</td>
<td>13 (21.7)</td>
<td>12 (23.1)</td>
<td>18 (24.0)</td>
<td></td>
</tr>
<tr>
<td>Afro-American</td>
<td>4 (6.0)</td>
<td>1 (1.7)</td>
<td>2 (3.8)</td>
<td>5 (6.7)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.4 ± 9.5 (range 20–70)</td>
<td>41 ± 9.3 (range 20–61)</td>
<td>45.3 ± 2.5 (23–74)</td>
<td>53.8 ± 12.1 (26–77)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Smoking, n (%)(^{2})</td>
<td>5 (7.5)</td>
<td>3 (5.1)</td>
<td>10 (20.4)</td>
<td>19 (26.0)</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)(^{2})</td>
<td>1 (1.5)</td>
<td>1 (1.7)</td>
<td>7 (14.3)</td>
<td>19 (26.0)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Healthy patients (no renal or periodontal disease).

\(^{1}\) With CP and without CKD.

\(^{2}\) Without CP and with ESRD.

\(^{3}\) With CP and with ESRD.

\(^{4}\) Chi-squared.

\(^{5}\) Mean ± standard deviation.

\(^{6}\) ANOVA.

\(^{7}\) Group 3 (n = 49) and group 4 (n = 73).
Table 2 - Periodontal status of the study population.

<table>
<thead>
<tr>
<th>Periodontal indices</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 67)</td>
<td>(n = 60)</td>
<td>(n = 52)</td>
<td>(n = 75)</td>
<td></td>
</tr>
<tr>
<td>Gingival Index</td>
<td>0.2 ± 0.4</td>
<td>1.6 ± 1.0</td>
<td>0.4 ± 0.6</td>
<td>0.8 ± 0.9</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Plaque Index</td>
<td>0.3 ± 0.4</td>
<td>1.3 ± 1.0</td>
<td>0.6 ± 0.9</td>
<td>1.1 ± 1.0</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Calculus Index</td>
<td>0.2 ± 0.2</td>
<td>0.9 ± 0.9</td>
<td>0.4 ± 0.8</td>
<td>0.8 ± 0.9</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.5</td>
<td>1.3 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>1.5 ± 0.1</td>
<td>2.3 ± 0.9</td>
<td>1.3 ± 0.6</td>
<td>2.4 ± 1.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mobility (absence/presence)</td>
<td>65/2</td>
<td>35/24</td>
<td>37/12</td>
<td>37/34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Healthy patients (no renal or periodontal disease).
2 Without CP and without CKD.
3 Without CP and with ESRD.
4 With CP and with ESRD.
5 Mean ± standard deviation.
6 Probing pocket depth.
7 Clinical attachment loss.
8 ANOVA.
9 Chi-squared.

The following primer pair was used for PCR amplification of genomic DNA samples for the MMP1-1607 (rs1799750) gene polymorphism: (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 of PCR Supermix (Go Taq Green Master Mix, Promega, Madison, WI, USA) and 1 μL of each 25 μM primer. The reactions were performed in a thermal cycler (Techne T-512, Barloworld Scientific US Ltd., Burlington, NJ, USA) and consisted of an initial denaturation step of 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 7 min.

A 10-μL aliquot of PCR products was mixed with a solution (New England Biolabs, Inc., Beverly, MA, USA) containing 2 μL 10× NE Buffer (50 mM NaCl, 10 mM Tris·HCl, 10 mM MgCl2, 1 mM diithiothreitol, pH 7.9), 0.02 μL bovine serum albumin (BSA, 10 mg/mL), 0.3 μL XmnI (20 units/mL) and 8 μL sterile deionised H2O. The solution was incubated at 37 °C overnight. Two mismatches were introduced in the reverse primer that annealed in the proximity of the polymorphism, which created a recognition sequence (5'-GAANNNTTC-3') for the restriction endonuclease XmnI (Invitrogen, San Diego, CA, USA) when the DNA template contains 1G (but not 2G) at the polymorphism site. Thus, XmnI digests the 1G allele, creating two fragments of 89 bp and 29 bp.

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

2.5. Analysis of MMP1 gene transcripts by real-time PCR

The pattern of mRNA expression for the MMP1 gene was investigated by real-time PCR and associated with genotypes in the entire population and within each group. From each group, 15 patients (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). TRizol (Invitrogen, San Diego, CA, USA) extraction of total RNA from periodontal tissues samples and cDNA synthesis were accomplished as previously described.

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, Foster City, CA, USA) for quantitation of amplicons as previously described. The standard PCR conditions were 95 °C (10 min), followed by 40 cycles of 94 °C (1 min), 56 °C (1 min) and 72 °C (2 min), and a standard denaturation curve. Real-time PCR conditions for each target were optimised with respect to the primer concentration, the absence of primer-dimer formation, and the efficiency of amplification of the target genes and the housekeeping control gene. The SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM of specific primers [MMP1 sense TGGACCTGGAGAATCTTGC, anti-sense AGATCAGAGAATGCGCA; β-actin: sense ATGGTGAGACCTTCAACA, anti-sense CCCGTCAGACTTCAATGAG] and 2.5 ng of cDNA were used in each reaction, as previously described. 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 PCRs was determined based on the negative controls (reactions performed without RNA and without reverse transcriptase). The mean Ct values from duplicate measurements were used to calculate the expression of the target gene, normalised to an internal control (β-actin), using the 2-(ΔΔCt) formula.

2.6. Statistical analysis

The significance of the differences in the observed frequencies of polymorphism between the groups was assessed by standard chi-squared (χ²) tests. Comparisons between the two groups for nominal variables in 2 × 2 tables were
Table 3 - General clinical aspects of ESRD patients.

<table>
<thead>
<tr>
<th></th>
<th>Group 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group 4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 52)</td>
<td>(n = 75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HD&lt;sup&gt;1&lt;/sup&gt; treatment (months)</td>
<td>43.2 ± 41.2</td>
<td>45.4 ± 42.3</td>
<td>0.783</td>
</tr>
<tr>
<td>General medical condition, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (14.3)</td>
<td>19 (26.0)</td>
<td>0.176</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>9 (18.3)</td>
<td>20 (27.4)</td>
<td>0.285</td>
</tr>
<tr>
<td>CVD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 (20.4)</td>
<td>18 (24.6)</td>
<td>0.664</td>
</tr>
<tr>
<td>Hypertension</td>
<td>31 (63.3)</td>
<td>61 (83.5)</td>
<td>0.017&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Current medication, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>35 (71.4)</td>
<td>55 (75.3)</td>
<td>0.678</td>
</tr>
<tr>
<td>Diuretics</td>
<td>11 (22.4)</td>
<td>30 (41.1)</td>
<td>0.050</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>34 (69.4)</td>
<td>52 (71.2)</td>
<td>0.842</td>
</tr>
<tr>
<td>Vitamin D (calcitriol)</td>
<td>9 (18.3)</td>
<td>12 (16.4)</td>
<td>0.810</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>2 (4.1)</td>
<td>9 (12.3)</td>
<td>0.196</td>
</tr>
<tr>
<td>Others</td>
<td>40 (81.6)</td>
<td>59 (80.8)</td>
<td>1&lt;sup&gt;o&lt;/sup&gt;</td>
</tr>
<tr>
<td>Habits, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>10 (20.4%)</td>
<td>19 (26.0)</td>
<td>0.522&lt;sup&gt;o&lt;/sup&gt;</td>
</tr>
<tr>
<td>Laboratory measurements&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP&lt;sup&gt;d&lt;/sup&gt; (mg/L)</td>
<td>7.7 ± 10.4</td>
<td>12.7 ± 17.9</td>
<td>0.030&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum creatinine (mg/L)</td>
<td>10.1 ± 2.7</td>
<td>9.8 ± 2.8</td>
<td>0.572</td>
</tr>
<tr>
<td>Normal protein catabolic rate</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.804</td>
</tr>
<tr>
<td>K/VD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.153</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>8.9 ± 0.7</td>
<td>9.1 ± 0.7</td>
<td>0.229</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dL)</td>
<td>5.3 ± 1.1</td>
<td>6.3 ± 1.2</td>
<td>0.000&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum calcium-phosphorus product (mg/dL)</td>
<td>50 ± 12.7</td>
<td>51.5 ± 12.8</td>
<td>0.509</td>
</tr>
<tr>
<td>Serum potassium (mg/dL)</td>
<td>5.2 ± 0.5</td>
<td>5.3 ± 0.6</td>
<td>0.579</td>
</tr>
<tr>
<td>PTH&lt;sup&gt;f&lt;/sup&gt; (pg/mL)</td>
<td>493.8 ± 593.1</td>
<td>468.7 ± 557.6</td>
<td>0.739</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td>131.7 ± 128.6</td>
<td>124.3 ± 107.1</td>
<td>0.793</td>
</tr>
<tr>
<td>Serum albumin (mg/dL)</td>
<td>3.7 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>0.260</td>
</tr>
<tr>
<td>Ferritin</td>
<td>700.7 ± 291.0</td>
<td>710.1 ± 299.1</td>
<td>0.865</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>6756.9 ± 1402.7</td>
<td>7108.1 ± 2336.0</td>
<td>0.308</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>11.5 ± 1.4</td>
<td>11.3 ± 1.5</td>
<td>0.637</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>34.7 ± 4.3</td>
<td>34.1 ± 4.6</td>
<td>0.503</td>
</tr>
</tbody>
</table>

Note: Missing data were not included.
<sup>a</sup> Chronic periodontitis.
<sup>b</sup> Chronic kidney disease.
<sup>c</sup> Haemodialysis.
<sup>d</sup> Cardiovascular disease.
<sup>e</sup> C-reactive protein.
<sup>f</sup> Marker of dialysis adequacy.
<sup>g</sup> Serum intact parathormone.
<sup>1</sup> Mean ± standard deviation.
<sup>+</sup> Fisher’s test.
<sup>**</sup> Mann–Whitney U test.
<sup>o</sup> Student’s t test.

performed using Fisher’s exact test. Statistical analysis was performed using statistical software (BioEstat 2.0 for Windows and Statistical Package for the Social Sciences 10.0 for Windows, Inc., Chicago, IL, USA). For continuous variables, Student’s t tests were used to compare the means of two groups. For non-parametric variables, the Mann–Whitney U test was used to assess differences between the groups. Continuous variables were expressed as the mean and standard deviation, and comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey’s test. The normal condition of the variables in each group was evaluated using the Shapiro–Wilks test. All statistical tests for gene transcripts were performed on graphic software (GraphPad InStat 3.05 and GraphPad Prism 3.0 software, GraphPad Software, Inc.). For all the tests, values of p < 0.05 were considered statistically significant.

3. Results

3.1 Genotyping analysis

The genotype distribution for the MMP1-1607 polymorphism was consistent with the assumptions of Hardy–Weinberg equilibrium. There was no statistically significant difference in the MMP1 polymorphism genotype distribution (p = 0.348) between the groups or in the allele frequency (p = 0.397) for the polymorphism studied. Genotypic frequencies and allelic distribution are shown in Table 4. When group 1 (control) vs. group 2 (patients with CP) was examined, no significant difference was noted for both the genotypic (p = 0.335) and allelic (p = 0.956) distributions. The same was true for the group 1 (control) vs. group 3 (patients with ESRD) genotypic (p = 0.145) and allelic (p = 0.212) frequencies as well as for the
group 1 (control) vs. group 4 (patients with ESRD and CP) genotype \( (p = 0.582) \) and allele \( (p = 0.359) \) distributions.

### 3.2. Genotyping and CP clinical parameters

With respect to the clinical periodontal status, the MMP1-1607*1G allele was associated with a higher mean PI in individuals without CKD (groups 1 and 2) \( (p = 0.045) \). In addition, an association between the 1G allele with PPD \( (p = 0.053) \) and CAL \( (p = 0.042) \) in ESRD patients (groups 3 and 4) was observed.

### 3.3. Genotyping and ESRD clinical parameters

In ESRD patients (groups 3 and 4), higher levels of two serum markers were associated with the MMP1*2G allele: ALT \( (p = 0.026) \) and calcium \( (p = 0.014) \). The MMP1*1G allele was associated with higher levels of ferritin \( (p = 0.030) \).

With respect to clinical aspects of ESRD, an association \( (p = 0.003) \) between the MMP1*2G allele and hepatitis was observed.

### 3.4. Analysis of MMP1 gene transcripts

There was an augmentation in the expression of the MMP1 gene in groups with CP (groups 2 and 4). Although the levels of expression were higher in group 4 compared with group 2, a statistically significant difference was not observed (Fig. 1).

No statistically significant difference was observed in relation to the MMP1-1607 genotype distribution in the entire sample. In group 4, there was an increase in MMP1 transcripts in the presence of the 2G allele, but no statistically significant difference was observed (Fig. 2).

### 4. Discussion

Matrix metalloproteinases play an important role in both the degradation and remodelling of ECM proteins during different physiological and pathological processes. At physiological conditions, TIMPs are in balance with the MMPs, and the ECM is remodelled 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 observed in some inflammatory diseases, such as CP and CKD.

In the present study, no significant difference was observed in the genotype distribution or in the allele frequency for the MMP1-1607 polymorphism between the groups. One limitation of this study was the small number of individuals in each group; genetic studies are more powerful as the sample size increases. Small samples could be a major cause of “negative results” in the experimental analysis, and if the N is not high enough to support definitive statements, the findings must be replicated in other populations. Another limitation might be the diagnosis of chronic periodontitis based only on clinical parameters, as radiographs were not used to define phenotypes in this study, and different manual probe pressures between examiners may affect the accuracy and reproducibility of diagnoses.

MMP1-1607 is a functional polymorphism, and there are a small number of studies investigating its association with CP. The MMP1-1607 polymorphism was investigated for CP in Asian populations. No association of the polymorphism with CP in Japanese patients was reported, although the 2G/2G
genotype, 2G allele frequency and 2G carriage rate were increased in cases of severe CP.47 Moreover, the frequency of the 2G allele and the 2G/2G genotype was higher in subjects with severe periodontitis in a Chinese population.48 There has been some discrepancies in the results of different analyses of this polymorphism in Brazilian populations. Astolfi et al.49 observed no association of genotypes and alleles of the MMP1-1607 polymorphism with CP groups. However, a different study reported an association of the MMP1'2G allele with severe CP.50 More recently, two studies in Turkish populations showed different results. Pirhan et al.51 reported an association of the MMP1'2G allele with severe periodontitis, and no association was found of this allele with periodontitis in the study by Ustun et al.52 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, gene-environment interactions, and a poor sample selection of extreme phenotypes.53 In fact, we observed that most studies considering extreme phenotypes (especially severe diseases) were able to detect an association between the MMP1-1607 gene polymorphism and CP.33,48,50 Thus, these data may indicate that the MMP1-1607 gene polymorphism is associated with CP severity rather than susceptibility.

No difference was observed in the frequency of genotypes and alleles between ESRD and control patients. It was observed in Japanese subjects that the MMP1-1607'1G allele was significantly associated with CKD53 and with CKD in individuals with a low serum concentration of HDL-cholesterol.54

Interestingly, with regard to periodontal indices, we found that the MMP1'1G was associated with PI in groups without CKD. Based on the presence of both periodontal bacteria and interleukins (e.g., IL-1, IL-6), which regulate MMP-1 expression, an association of the MMP1'2G allele with higher means of PI might be expected; however, dental biofilm represents a more favourable environment for the initiation rather than the progression of CP.55 Disease progression and severity may be further dependent on the immune-inflammatory host response to microbial challenge.56 MMP1'1G was also associated with higher means of PPD and CAL in ESRD patients, although the expected association was with the 2G allele. In an inflamed systemic environment, such as in ESRD patients, it is possible that some complex gene expression regulation may occur.

An association between the MMP1'2G allele and higher levels of both ALT and hepatitis was found. The transaminases, such as ALT, are considered to be “classical” serum markers of liver necro-inflammatory injury.56 It is recommended that patients on haemodialysis therapy, who constitute a risk group for hepatitis C virus (HCV) infection, submit to regular screening for HCV infection using ALT values.57 HCV infection is frequently asymptomatic in these patients and should be suspected in all patients presenting elevated ALT.58 The prevalence of HCV is significantly higher in haemodialysis and kidney transplant patients than in the general population.59 Chronic HCV infection remains an important cause of liver disease in patients with ESRD, and renal failure has a significant impact on morbidity and mortality throughout the natural history of chronic HCV.60 The MMP1'1G allele was also associated with higher levels of ferritin. Serum ferritin is a clinically important index of body iron stores, but its functional role remains largely obscure.61 When ferritin is also considered as an acute-phase reactant,62 inflammatory factors may interfere with the synthesis and clearance of ferritin, thereby increasing serum ferritin levels in a manner unrelated to the iron status.63 Higher levels of serum calcium were associated with the 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.64 The kidney plays a leading role in maintaining calcium and phosphorus homeostasis in collaboration with other organs, such as the parathyroid gland, intestines, and bones. Along with the progression of CKD, various abnormalities of mineral and bone metabolism may exist.65 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 in the vasculature, which results in cardiovascular complications and mortality.66 In addition, it was recently reported that the

Fig. 2 – Quantitative expression of the MMP1 gene in gingival tissues of patients from the four groups according to genotype: 1G/1G, 1G/2G, 2G/2G. Total RNA was extracted, and the levels of MMP1 were measured quantitatively using the real-time PCR SYBR-Green System. The results are presented as the fold increase of expression of the mRNA, normalised to β-actin, and compared with the target-internal control using the cycle threshold (Ct) method.
2G allele is associated with mortality in haemodialysis patients.\textsuperscript{57}

MMP1 gene transcripts were analysed in patients presenting CP and ESRD and in control patients. The levels of MMP1 transcripts were higher in the group with CP and ESRD (group 4) compared with the group with only CP (group 2), but no significant difference was observed. Excessive or inappropriate expression of MMPs has been associated with progressive renal injury, glomerular sclerosis\textsuperscript{23} and interstitial kidney fibrosis.\textsuperscript{26} Preston et al.\textsuperscript{38} 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 haemodialysis groups may be a reflection of gene stimulation induced by inflammatory cytokines. Moreover, in the group with both diseases, there was a progressive increase of MMP1 transcripts in the presence of the 2G allele. It was previously reported that an additional guanine (G) creates an Ets (a family of transcription factors)-binding site, promoting significantly increased transcription in normal fibroblasts, thereby providing a mechanism for more aggressive matrix degradation.\textsuperscript{30} Perhaps in the presence of a systemic disease, the association of the 2G allele with the augmentation of gene expression in CP is more evident. Furthermore, the presence of a high variation in MMP1 expression between individuals with the same genotype in groups with CP was noted. This could be due to the additional influence of specific periodontopathogens and cytokine stimulation.

Chronic infections, such as CP, appear to be significant causes of persistent systemic inflammation in patients with ESRD\textsuperscript{69} and, in turn, systemic conditions, such as ESRD, may impact CP outcome.\textsuperscript{70} Moreover, MMPs are overexpressed in inflammatory conditions, such as CP and ESRD, increasing the destruction of ECM components. Thus, investigating polymorphisms that interfere with the regulation of MMP expression may contribute to an understanding of common genetic mechanisms.

In summary, no evidence for the association of MMP1-1607 (1G/2G) with susceptibility to CP or ESRD was observed. However, other polymorphisms in MMP genes may have an impact on the response to CP and ESRD inflammatory insult. An augmentation of the levels of MMP1 gene transcripts in periodontally affected tissues of patients with and without CKD was observed.

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**Competing interests**

The authors declare no conflicts of interest related to the commercial products used in this study.

**Ethical approval**

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).

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