Prevalence of *Porphyromonas gingivalis* fimA II genotype in generalized aggressive periodontitis patients

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**Abstract**

**Purpose:** The objective of this study was to evaluate the prevalence of *Porphyromonas gingivalis* (Pg) and its fimA II genotype in a sample of Brazilian patients with generalized aggressive periodontitis (GAgP) and to correlate the presence of each pathogen/genotype with clinical parameters.

**Methods:** We used polymerase chain reaction (PCR) to evaluate the presence of *Pg* and fimA II genotype in subgingival plaque samples collected from the deepest site of 45 Brazilian patients aged 15-40 years with GAgP and correlated findings with age and clinical parameters (plaque index, gingival bleeding index, probing depth and clinical attachment loss).

**Results:** *Pg* was identified in 64.4% patients. FimA II genotype was present in 82.6% of *Pg*-positive patients. The presence of *Pg* and fimA II genotype was significantly associated with greater clinical attachment loss at the sampled periodontal site. *Pg*-positive patients were slightly older than *Pg*-negative patients.

**Conclusions:** *Pg* and fimA II genotype were highly prevalent in Brazilian patients with GAgP. *Pg* was more commonly observed in slightly older individuals and in sites with more clinical attachment loss.

**Keywords:** Aggressive periodontitis. Bacterial fimbriae. *Porphyromonas gingivalis*.

**Introduction**

Aggressive periodontitis (AgP) is a rapidly progressive form of periodontitis affecting systemically healthy patients¹. Less common and more severe than chronic periodontitis, AgP tends to run in families, with a preference for younger individuals². Such characteristics suggest the presence of highly virulent pathogens and/or a high level of susceptibility to the disease¹,². Over the past few years, much research has been conducted on the etiology and pathogenesis of AgP³,⁴.

Strong evidence exists for the role of *Aggregatibacter actinomycetemcomitans* (Aa), especially the JP2 clone, as an etiological factor in the pathogenesis of AgP⁵. However, in some populations *Pg* can also be associated to AgP³,⁴. This organism displays great genotypical and phenotypical diversity resulting in variations in virulence and ability to induce periodontal destruction⁶. The virulence of *Pg* is consistently associated with the presence of fimbriae, structures related to cell adhesion⁷. The fimbriae play an important role in the invasion and colonization of periodontal tissues⁸. Fimbrillin (fimA), a subunit protein of the fimbriae in this organism, is encoded.
by the fimA gene. Based on nucleotide sequence, six fimA genotypes have been identified: fimA I, Ib, II, III, IV and V. FimA II is the most prevalent in patients with periodontitis, usually followed by FimA IV and Ib. The genotypical diversity of fimA genotype has been evaluated for a range of periodontal conditions, but to our knowledge only two studies have focused on AgP. In these studies, AgP was strongly associated with genotype fimA II in Japanese and Chinese subjects.

Microbiological variations in AgP patients related to ethnicity and socio-demographic conditions justify conducting studies on different populations. In addition, by correlating the presence of periodontal pathogens with clinical parameters, individuals at risk may be identified. Thus, we evaluated the prevalence of Pg/fimA II genotype in generalized aggressive periodontitis (GAgP) Brazilian patients and correlated the presence of each pathogen/genotype with clinical parameters.

Material and methods

Selection of subjects

This study was conducted in 45 GAgP patients selected at the Periodontology Clinic of the School of Pharmacy, Dentistry and Nursing, at the Federal University of Ceará, Brazil. GAgP patients were classified according to the clinical criteria suggested by the American Academy of Periodontology: generalized interproximal attachment loss affecting at least three permanent teeth other than the first molars and incisors in systemically healthy individuals with rapid attachment loss and bone destruction. To be eligible for the study, patients had to be aged 12-40 years and have at least 20 teeth other than third molars, of which at least three first molars and five incisors. The clinical diagnosis was confirmed by evidence of inter-proximal bone loss on full-mouth periapical radiographs.

The exclusion criteria were as follows: periodontal treatment within the previous 6 months, antibiotic therapy within the previous 3 months, smoking habits and systemic changes capable of interfering with periodontal health.

The research protocol was approved by the Ethics Committee of the Federal University of Ceará, Brazil (no. 20/08). After being informed about the purpose of the study, all participants or guardians gave their written consent.

Clinical measurements

Clinical measurements were made on all the completely erupted permanent teeth, except the third molars, using a periodontal probe (PCP-UNC 15, Trinity, São Paulo, SP, Brazil). The following parameters were evaluated: plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment loss (CAL). PD and CAL were taken at six sites per tooth (mesiobuccal, bucal, distobuccal, mesiolingual, lingual, and distolingual). A single examiner evaluated all the clinical periodontal parameters, and measurement reproducibility was calculated by using the intraclass correlation coefficient (ICC) for PD and CAL. The agreement between replicate measurements was high (ICC > 0.80).

Microbiological sampling and evaluation

The supragingival plaque was removed with curettes and sterile cotton pellets, and the area was isolated with sterile cotton rolls. Subgingival plaque samples were collected by means of two sterile paper points (Dentsply Maillefer 35, Dentsply, Rio de Janeiro, RJ, Brazil) at the selected site for 20 seconds. The selected site was the proximal site with the greatest PD and CAL of molars or incisors of each patient. The samples were separately immersed in microtubes containing 1 mL Ringer’s sterile solution and stored at -80°C until they were processed.

Each sample was processed separately. The microtubes containing the samples were thawed on ice. The bacterial cells or suspension were dispersed by vortexing at the maximum setting for 1 min and centrifuged at 12,000x g for 10 min. Genomic DNA was extracted from the pellet (InstaGene Matrix, Bio-Rad Laboratories, Hercules, CA, USA) and a 20 μL aliquot of the resulting supernatant was added to 30 μL reaction mixture containing 25 μM PCR buffer (Promega Corporation, Madison, WI, USA), 25 μM MgCl₂ (Promega Corporation, Madison, WI, USA), 0.2 μM dNTP mix (Promega Corporation, Madison, WI, USA), 1.25 U Taq polymerase (Promega Corporation, Madison, WI, USA), and 100 ng of each primer (Invitrogen, São Paulo, SP, Brazil), resulting in a final volume of 50 μL. Negative and positive controls were included in each reaction.

First, PCR was performed with universal primers (5′-GGACTAYAGGGGTATCTAAT-3′; 5′-AGAGTTTGATCMTGG-3′) for the 16S ribosomal DNA (16S rDNA) to confirm the presence of bacterial DNA. Subsequently, the samples were evaluated by PCR with specific primers for the presence of Pg (5′-TGTAGATGACTGATGTTGGAACCC-3′; 5′-ACGTCATCCCAACCTTCC-3′) and fimA II genotype (5′-ACAATATATGTTAATGGAAG-3′; 5′-AACCCCCGTCCCTGGTATTCCGA-3′). The primers resulted in amplicons in 197 and 257 bp from strains of Pg (#HW24D-2) and genotype fimA II (#HW24D-2), respectively, which were used as controls.

Amplification (Biocycler, Biosystems, Curitiba, PR, Brazil) of the 16S rDNA was performed with an initial cycle at 94°C for 10 min, followed by 30 cycles at 96°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. For Pg, amplification was performed with an initial cycle at 95°C for 5 min, followed by 30 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 7 min. For fimA II genotype, amplification was performed with an initial cycle at 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 7 min.

The amplification products were analyzed by electrophoresis on agarose gel. A 1.5% gel was used to assess the 16S rDNA and the presence of Pg, while a 2.0% gel was used to assess the fimA II genotype. The gels were stained with SYBR® Safe (Invitrogen, São Paulo, SP, Brazil) and photographed (Canon Powershot A640, Canon, USA) under ultraviolet light (LTA/LTB GE, Locusc Bioteconomia, São Paulo, SP, Brazil). PCR was repeated three times for each sample and for each microorganism.

Statistical analysis

The normality of the data distribution was verified with the Kolmogorov-Smirnov test. Comparisons between clinical
data and bacterial findings were made with the non-paired t test (for normally distributed data) and the Mann-Whitney test (for non-normally distributed data). The level of significance was determined to be 5%. All data analyses were performed with the software GraphPad Instat (GraphPad Software Inc., San Diego, CA, USA).

Results

The sample included 45 subjects with GAgP aged 15-40 years (mean: 28.8). The clinical findings of the sample are presented in Table 1.

Pg was present in 29 patients (64.4%) and fimA II genotype in 25 (86.2%) of the 29 subjects testing positive for Pg. Table 2 shows mean periodontal findings according to the presence/absence of Pg and fimA II genotype. Statistically significant difference was found for age between Pg+ and Pg- patients. Mean clinical attachment loss was significantly greater at the sampled site for microbial analysis in Pg+ and also in fimA+ patients.

Table 1 - Clinical data of the GAgP patients.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age; age range (years)</td>
<td>28.8 ± 5.9; 15-40</td>
</tr>
<tr>
<td>Mean number of teeth</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Mean PD fm (mm)</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Mean CAL fm (mm)</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>Mean PD at sampled site (mm)</td>
<td>9.0 ± 1.9</td>
</tr>
<tr>
<td>Mean CAL at sampled site (mm)</td>
<td>9.9 ± 2.2</td>
</tr>
<tr>
<td>Mean GI (%)</td>
<td>12.7 ± 9.8</td>
</tr>
<tr>
<td>Mean PI (%)</td>
<td>32.1 ± 17.4</td>
</tr>
<tr>
<td>PD ≥5 mm (% of sites)</td>
<td>26.5 ± 14.5</td>
</tr>
<tr>
<td>PD ≥5 mm (% of teeth)</td>
<td>59.0 ± 20.6</td>
</tr>
<tr>
<td>CAL ≥5 mm (% of sites)</td>
<td>31.7 ± 17.0</td>
</tr>
<tr>
<td>CAL ≥5 mm (% of teeth)</td>
<td>65.2 ± 19.1</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. GAgP: generalized aggressive periodontitis; PD: probing depth; CAL: clinical attachment loss; GI: gingival index; PI: plaque index; fm: full mouth.

Discussion

This study presents results to support the idea that Pg play an important role in the etiology of AgP. It has been associated with AgP in certain populations. It was one of the most frequently detected species in AgP Chinese patients, harboring almost a hundred percent of the patients. In the current study Pg was detected in 64.4% of the subjects. In addition, fimA II genotype was detected in over 85% of patients colonized by Pg. Among the different Pg fimbriae genotypes, fimA II is the most virulent and has been related to periodontal disease progression.

AgP is often associated with Aa, however, it has been shown that the virulence of Aa decreases over time. There is evidence that the Aa leukotoxicity decreased in patients with localized aggressive periodontitis (LAgP) originally colonized by highly leukotoxic clones. Another study found an increased prevalence of Aa in shallow and intermediate pockets of LAgP patients only, whereas the prevalence of Pg was high in both LAgP and GAgP, suggesting Aa is predominantly associated with LAgP while Pg is associated with the progression of LAgP and with GAgP. Once the patients of the current study presenting Pg are older and presented sites with greater periodontal destruction as well as the ones presenting the fimA II genotype, it can be suggested that over time P. gingivalis can play an important role in AgP progression.

The prevalence of Pg observed in this study (64.4%) was lower than the prevalence generally reported in the literature. In three Brazilian studies, Pg was found in 80%, 86.7% and 73.3% of AgP patients. In Chile and in Japan, the prevalence was 88.8% and 79.8%, respectively. In China, it was even higher, colonizing nearly 100% of a sample of Chinese AgP patients. The smaller prevalence of Pg found in the present study also may be related by the fact that our samples were collected from the site of greatest probing depth in each patient, whereas the above-cited studies pooled samples from multiple sites. However, a study comparing samples from one against three deepest periodontal sites, evaluated by molecular biology technique, concluded that there was no difference for the frequency of Pg.

To our knowledge, only two studies reported prevalences for Pg fimA genotypes in patients with AgP. The prevalence of fimA II genotype observed in this study may be considered high (86.2%) compared to another results, that evaluated the prevalence of six genotypes in AgP patients and found fimA II to be the most common type: 40.5% and 47.3%. These two studies analyzed patients with characteristics similar to ours, including mean age and periodontal destruction. However, while the number of AgP patients recruited was greater to Feng et al., who studied 81 Chinese patients, Miura et al. analyzed only 18 Japanese patients.

FimA II genotype is considered particularly virulent due to its association with periodontal destruction. FimA II adheres to and invades human epithelial cells more efficiently than other genotypes. Studies also demonstrated that fimA II genotype can induce a stronger inflammatory reaction. In addition, when comparing Pg genotypes in periodontitis patients with diabetes after periodontal treatment, fimA II was detected only in subjects with increased levels of glycated hemoglobin (HbA1c), while improvements in HbA1c values were observed in subjects without...
type II clones, suggesting that glycemic levels in diabetes are affected by the persistence of \( Pg \ fimA \) II genotype\(^{26}\). These studies suggest that \( fimA \) II genotype is an important factor of virulence in \( Pg \) and may play a role in inflammatory response not only in periodontitis, but also in systemic diseases\(^{26,29}\).

In our study, the presence of \( Pg \) and genotype \( fimA \) II was associated with greater clinical attachment loss at the site sampled for microbial analysis. These findings are supported by studies that observed a higher prevalence of \( Pg \) at sites with greater attachment loss in patients with chronic periodontitis\(^{4,13}\), and that founded the prevalence of \( fimA \) II genotype to be greater in deeper periodontal pockets\(^{26}\). Therefore, the presence of this genotype may be used to identify individuals at risk, establish plan treatments and prognosis. These findings can also be useful in the development of novel treatment strategies, such as passive immunotherapy\(^{30}\).

In conclusion, in the present sample of Brazilians with GAgP, the prevalence of \( Pg \) and \( fimA \) II genotype was high. Additionally, \( Pg \) was more prevalent among slightly older patients and at sites with greater periodontal destruction, and \( fimA \) II genotype was more present in sites with greater clinical attachment loss.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

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**References**

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