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ORIGINAL ARTICLE

Distribution of *Porphyromonas gingivalis fimA* genotypes in patients affected by rheumatoid arthritis and periodontitis

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ABSTRACT

Objective: To determine and compare the distribution of *Porphyromonas gingivalis fimA* genotypes in patients affected by Rheumatoid arthritis (RA) and periodontitis (PE).

Materials and methods: This study involved 394 subjects divided into four groups, RA, PE, RA and PE and healthy subjects. PE was diagnosed by using clinical attachment loss (CAL) and probing depth (PD) indexes. Presence of *P. gingivalis* and its genotypes was identified by polymerase chain reaction in subgingival biofilm.

Results: *P. gingivalis* was more frequent in patients with RA (82.69%), and *fimA* II genotype was the most frequent in all groups, especially in PE/RA (76.71%). There was statistical difference ($p < .05$) regarding the frequency of *P. gingivalis* genotypes such as *fimA* Ib, II and III.

Conclusions: Distribution of *P. gingivalis fimA* II genotypes was different among groups, it could play a critical role in the presence of PE in RA patients.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of synovial joints with a prevalence of 0.5–1% worldwide [1]. RA physiopathology is well recognized, but its aetiology is still unknown being viral and bacterial infections involved as possible trigger factors specially periodontal bacteria, such as *Porphyromonas gingivalis* have been considered. *P. gingivalis* is the only known pathogen expressing peptidylarginine deiminase (PPAD), an enzyme responsible of citrullination process, that produce citrullined antigens driving adaptative immune responses that are nearly exclusive to RA [2].

On the other side, periodontitis (PE) is a chronic inflammatory disease characterized by gingival inflammation and alveolar bone destruction leading to tooth mobility and loss [3]. There are several studies that have concluded that patients with RA are more likely to have PE [4–7], and many clinical and pathologic features are shared between RA and PE [8]. The main periodontal bacterium *P. gingivalis* has several virulence factors, including: lipopolysaccharide, hemagglutinin, haemolysin, extracellular proteinases and fimbriae [9].

Fimbriae are hair-like appendages involved in adherence, invasion and pro-inflammatory activity [9,10]. In basis of *fimA* gene which encodes fimbriline, the structural protein of fimbriae, *P. gingivalis* has been classified into six genotypes (I–V, and Ib) [11,12]. Many studies have shown that clones of *fimA* genotypes Ib, II and IV are significantly more aggressive, such *in vitro* and *in vivo* conditions compared with other genotypes and they could play an important role in the adhesion to host tissues and progression of PE, while types I and III are considered less virulent [13].

However, there are reports about distribution of *P. gingivalis fimA* genotypes in different systemic diseases as diabetes mellitus, there are no reports in RA patients, therefore, the aim of the present study was to determine and compare the distribution of *fimA* genotypes of *P. gingivalis* in RA patients affected by PE.

Materials and methods

Subject population and clinical evaluation

This cross-sectional study was performed from December 2016 to March 2017 involving 394 subjects recruited by a non-probabilistic consecutive sampling. The subjects were selected from the Regional Unit of Rheumatology and Osteoporosis at the Central Hospital and at the Oral Medicine Clinic of the Master's Degree in Advanced General Dentistry Program at San Luis Potosi University, San Luis Potosi, Mexico.

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They were divided into four groups: Control ($n = 89$), PE ($n = 73$), RA ($n = 104$), and PE/RA ($n = 128$). Patients included a history of at least five-year from initial RA diagnosis performed by a Rheumatologist applying criteria of the American College of Rheumatology and European League Against Rheumatism established in 2010 [14]. PE diagnosis was performed by a calibrated examiner. The inter-examiner consistency was 0.85. Clinical periodontal parameters were examined in all patients: probing depth (PD) and clinical attachment loss (CAL) indexes were assessed using a North Carolina periodontal probe (Hu Friedy, Chicago, IL, USA) graduated in millimeters (0–15mm). The probe was inserted parallel to teeth long axis and crossed each tooth's surface circumferentially. CAL index was measured from the epithelial attachment to the cement-enamel junction. Generalized PE diagnosis was determined based on the amount of CAL and is designated as moderate (3 or 4 mm) in more than 30% of the teeth were involved [15].

All patients who had received previous periodontal treatment and antibiotic therapy within the last three months were excluded; the subjects that were included signed a written informed consent. The study was approved by the research Committee of Master's Degree Program in Advanced General Dentistry at San Luis Potosi University, Mexico.

Subgingival plaque sample

After cleaning the teeth's crown with a sterile sponge, subgingival dental plaque (SDP) was taken with a Gracey curette and placed into a Eppendorf tube with 1 ml of phosphate buffer saline (PBS). Samples were stored at −80 °C until the DNA extraction.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR)

Plaque samples were washed with PBS. DNA was extracted by phenol–chloroform purification and isopropanol precipitation method as previously described [16]. DNA was rehydrated by Tris-EDTA and the concentration obtained was determined by spectrophotometry (Nanodrop 2000; Thermo Scientific, Madison WI, USA). The presence of *P. gingivalis* was detected by PCR. DNA sequences in acquired plaque samples were identified using *P. gingivalis*-specific sequences, and analysed further to differentiate their *fimA* genotypes using the six sets of *fimA* genotype-specific primers (Table 1). DNA amplification was performed with a thermal cycler (iCycler; BIO-RAD Laboratories, Hercules, CA, USA) with cycling protocols specific for each set of primers [12]. Positive and negative controls were included in each PCR set. The PCR products were analysed by electrophoresis in a 2% agarose gel–Tris–acetate EDTA buffer, and a 100 bp DNA ladder marker (New England Biolabs, Beverly, MA, USA) was used as a reference for molecular size [11]. Gels were stained with 0.5 μg of ethidium bromide/ml and observed under UV light (E-Gel Imager System with UV Base; Thermo Fisher Scientific, Life Technologies, Waltham, MA, USA).

Statistical analysis

The examiner was calibrated in PE diagnosis by an expert using an intraclass correlation coefficient test. Qualitative data are expressed as frequency and proportion; quantitative data are expressed as mean, standard deviation and range. For determination of data distribution, a Kolmogorov–Smirnov test was applied. To detect statistical differences among groups for quantitative variables, a Kruskal–Wallis and ANOVA (analysis of variance) tests were employed. For gender and frequency of genotypes, Fisher's exact test was applied. Statistical significance was set at $p < .05$ employing SPSS 18.0 (IBM, Chicago, IL, USA).

Results

The mean age of the study population was around 50 years old and the gender was predominantly female (90%), there was not statistical differences, therefore four groups are homogeneous (Table 2). As expected, comparing between RA and control groups similar means of full mouth measurements about PD (2.42 ± 0.49 vs 2.2 ± 0.42 mm, $p > .05$) and CAL indexes (1.86 ± 0.52 vs 1.29 ± 0.36 mm, $p > .05$) were obtained. On the other side, comparing PE/RA and PE groups they obtained similar levels of PD (3.2 ± 0.68 and 3.6 ± 1.17mm $p > .05$) and CAL (2.54 ± 0.46, and 3.12 ± 0.58 mm $p > .05$) (Figure 1).

Regarding the *P. gingivalis* and *fimA* genotypes distribution (Figure 2), shows that there are statistical differences

Table 1. Specific oligonucleotides used in this study.

Primer	Sequence	Amplicon (bp)	Reference
P. gingivalis	F: TGTAGATGACTGATGGTGAAAACC R: ACGTCATCCCCACCTTCCTC	197	Amano et al. [25]
fimA I	F: CTGTGTTTTATGGCAAACTTC R: AACCCCGCTCCCTGTATTCCGA	392	Amano et al. [25]
fimA Ib	F: CAGCAAGACCAAAAACAATCG R: TGTCAGATAATTAGCGTCTGC	271	Nakagawa et al. [12]
fimA II	F: GCATGATGGTACTCCTTTGA R: CTGACCAACGAGAACCCACT	292	Moon et al. [26]
fimA III	F: ATTACACCTACACAGGTGAGGC R: AACCCCGCGCCCGCTATTCCGA	247	Amano et al. [25]
fimA IV	F: CTATTCAGGTGCTATTACCCAA R: AACCCCGCTCCCTGTATTCCGA	251	Amano et al. [25]
fimA V	F: AACAACAGTCTCCTTGACAGTG R: TATTGGGGGTCGAACGTTACTGTG	462	Nakagawa et al. [12]

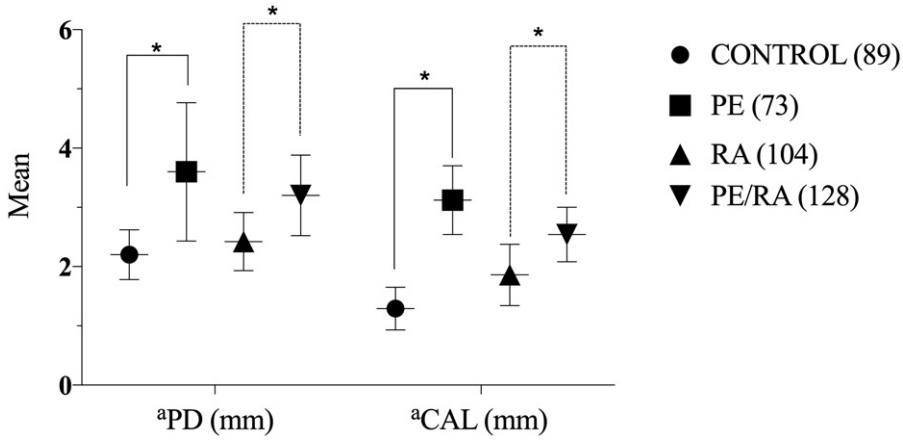

Figure 1. Periodontl Status. Mean values of Pocket Depth (PD mm) and Clinical Attachment Loss (CAL mm). PE and PE/RA groups showed higher means about PD and CAL vs Control and RA groups respectively; $^{*}p < .05$; ANOVA test.

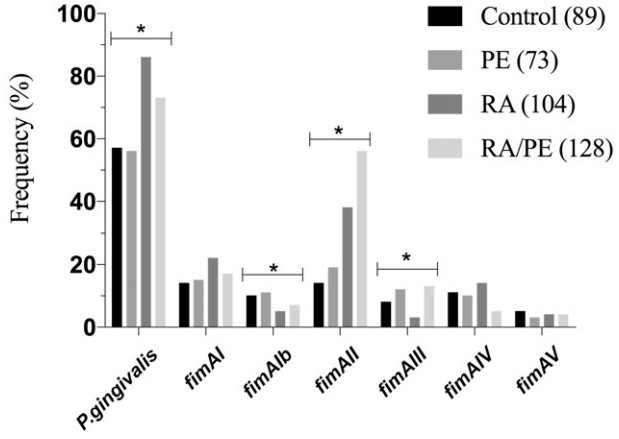

Figure 2. *P. gingivalis* and *fimA* genotypes distribution. *P. gingivalis* and *fimA* Ib, III genotypes, showed statistical differences among groups $^{*}p < .001$; fimA II genotype considered the most virulent has the highest frequency in RA/PE group $^{*}p < .0001$; PE: Periodontitis; RA: Rheumatoid Arthritis; *Fisher test.

among groups ($p < .0001$), being RA the group with the highest frequency of *P. gingivalis* (82.7%).

Comparing the frequency of *P. gingivalis fimA* genotypes: Ib, II and III there was significant statistical differences among the four groups ($p < .05$) being genotype *fimA* II the most frequent in all groups, especially in PE/RA group (76.7%) (Table 2).

Discussion

Epidemiological studies have shown the relationship between PE and RA, where *P. gingivalis* bacterium plays an important role [8]. This study examined the frequency of *P. gingivalis fimA* genotypes in subgingival plaque samples from patients with PE and RA by PCR. Some case-control studies that involved healthy subjects and RA patients showed a positive association between PE and RA ($p \leq .05$; OR = 8.05) [5], and other reports have studied the potential role of *P. gingivalis* in RA pathogenesis [17]. Some authors found a high frequency of *P. gingivalis* in RA and PE (35%) [2]. In this study, all subjects were selected from a homogeneous population with similar age and gender. Also, we employed strict criteria to define PE (30% of probed sites affected with PD > 3 mm and ≥ CAL 2 mm) [15]. PE and PE/RA were similar about periodontal status, therefore, the population of the current study is very suitable for the investigation about *P. gingivalis* genotypes *fimA* distribution.

It was unexpectedly observed that the frequency of *P. gingivalis* in subgingival dental plaque in the PE/RA group had the lowest frequency (57%) compared to the other groups. Our results suggest that *P. gingivalis* is not essential for the presence of PE in this population, probably the immune condition in RA patients and other periodontal species such as *Tannerella forsythia* could have a role in developing of PE in RA patients because some reports indicated a high prevalence of this bacterium (65.3%) [18]. However, *P. gingivalis* had six different genotypes based in the sequence of *fimA* gen that codifies for fimbriline, the structural protein of fimbriae, one of the virulence factors of *P. gingivalis* [11]. These genotypes confer different pathological capacities and it could be the possible explanation of the high prevalence of PE in patients with RA.

There are some reports that affirm that *P. gingivalis fimA* genotypes Ib, II and IV are the most virulent variants involved in PE and contribute in adhesion and invasion of periodontal tissues. In contrast, in healthy subjects *fimA* I was the most prevalent genotype [11,18,19]. In a multiracial population the most predominant *P. gingivalis* genotype was *fimA* II in PE patients, such as the one in the Brazilian study, which agreed with our results. This data strongly suggested that is important to consider ethnic and demographic variables when final conclusions among cross-population studies are being drawn. It is important to know which genotype of *P. gingivalis* is directly associated with presence of PE in patients affected by RA. Additionally, it was suggested that in some cases, *P. gingivalis* colonization is a contributory but non-essential factor for the progression of periodontal disease [20]. Although differences among studies exist, type II *fimA* has been found to be the most closely related to chronic PE; this corresponds to the results of the present study based on a RA population sample, in which the highest detection rate of type II *fimA* was observed in patients with PE/RA. In this study the control group revealed a similar prevalence for *fimA* I and II, these

Table 2. Clinical features of study subjects.

Group	Control ($n = 89$)	PE ($n = 73$)	RA ($n = 104$)	PE + RA ($n = 128$)
		X ± SD (Range)		
Age (years)*	50.54 ± 11.33 (30–74)	51.04 ± 12.53 (30–80)	49.75 ± 11.94 (21–81)	51.96 ± 11.67 (21–88)
		Frequency (%)		
Gender**				
Male	15 (16.85)	12 (16.43)	10 (9.61)	11 (8.59)
Female	74 (83.15)	61 (83.56)	94 (90.38)	117 (91.40)

PE: Periodontitis; RA: Rheumatoid arthritis; X: mean; SD: Standard Deviation.
*Kruskal–Wallis $p > .05$.
**Fisher test $p > .05$.

results are similar to Colombian reports that have a high prevalence of *fimA* II in healthy patients [18], and differ from other studies that report low frequencies of this genotype and high frequencies of *fimA* I, III and V, considered as pathogens with a low aggressive capacity [11,21,22]. These findings can explain the possibility that the most virulent genotypes present in the periodontal disease initiation such as a gingivitis or PE, facilitate the process of colonization an infection, and stimulate the inflammatory and immune response with time could cause tissue damage and destruction. In this study, the PE/RA group has the highest prevalence of *fimA* II (76.7%). It could explain that type II in patients with RA was capable of more efficient attachment to host cells and cell invasion, to playing an important role in the pathogenesis of PE in patients with RA. In this population our results are agree with the majority of studies, indicating that type II is the predominant genotype in patients affected by PE [23,24] causing invasion and adhesion, as well as a pro-inflammatory process that lead the destruction of oral tissue and loss of teeth in patients with PE/RA. We concluded that *P. gingivalis* is most frequent in patients with RA than PE/RA, but the genotype *fimA* II was more frequently detected in PE/RA, and this could be the explanation of the high prevalence of PE in patients with RA.

Disclosure statement

No potential conflict of interest was reported by the authors.

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