

Occurrence of *Porphyromonas gingivalis* in patients with periodontitis in Brazil

Ocorrência de *Porphyromonas gingivalis* em pacientes portadores de periodontite no Brasil

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ABSTRACT

The aim of this study was to evaluate the occurrence of *Porphyromonas gingivalis* in patients with periodontitis in Brazil and investigate whether there is a relationship between your occurrence and gender and age. Analysis was performed of 1,386 results of microbiological examinations of samples of subgingival microbiota in patients with periodontitis. Collection was performed by periodontists in private practice and processed by culture in clinical laboratory of oral microbiology. The chi-square test was used to verify the association of occurrence of *P. gingivalis* with gender and age. It was observed that 59% of examinations were of female patients and 41% were of male patients, most of which aged more than 40 years (64.3%). The occurrence of *P. gingivalis* was 17.8%. There was no significant difference in the presence of this bacterium in the different age ranges, yet there was a significantly higher occurrence in males. It was observed that, regardless of the age range, the occurrence of *P. gingivalis* was always significantly higher in males. It was concluded that the occurrence of *P. gingivalis* in patients with periodontitis in Brazil was 17.8% and was associated with gender, yet had no association with the age range.

UNITERMS

Porphyromonas gingivalis; periodontal diseases, disease occur measures, male, female, middle aged; microbiological analysis

INTRODUCTION

The prevalence and severity of periodontal disease shows similarities and variations in different populations⁸. Such variations may be due to different oral hygiene levels or may reflect racial or ethnic factors that could modify the composition of subgingival microbiota and/or the hosts' answer^{9,15,26}. In some populations, it was observed that, although individuals presented poor oral hygiene, there was no presence of advanced periodontal disease⁸. Thus, there may be differences in the microbiota associated with periodontal disease in different populations²⁸. Many studies have been conducted in European and

North American populations in an attempt to relate specific bacteria to periodontal disease²⁷. Little is known about the general demographic distribution of the microbiota associated with periodontal disease. Recently, studies have been conducted in developing countries^{2,4,5,11,16,21,28} in an attempt to relate specific bacteria to periodontitis.

The bacterial etiology of periodontal disease is already established; however, only few bacteria in the oral cavity are probably directly involved in the initiation and progression of periodontal disease. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* were recognized as true periodontal pathogens¹².

P. gingivalis is a major putative pathogen in adult periodontitis^{5,11}, has been associated with generalized juvenile periodontitis¹, and it is implicated in rapidly progressive periodontitis. It is detected in significantly higher occurrence and proportions in progressive lesions than in non-progressive lesions²⁷.

Monitoring of these bacteria could help identifying sites at risk for progression of periodontal disease, selecting antimicrobial agents and assessing the treatment²⁷.

In this context, the purpose this study was to assess the occurrence of *P. gingivalis* and its association with sex and age in patients with periodontal disease in the Brazilian population.

MATERIAL AND METHODS

Results of microbiological examinations of subgingival samples of periodontal patients from the Brazilian population were analyzed. These exams were performed by periodontists in private clinics and sent to the Oral Microbiology Clinical Laboratory (Periolab Microbiological Analysis, SP, Brazil) during the period 1997-2002. The material and steps followed for sample collection were established by instructions supplied by the laboratory. This study was approved by the Ethics Committee of the Dental School of University of São Paulo.

Selection criteria required microbiological samples collected from periodontal sites characterized by probing pocket depth >4 mm with bleeding on probing or suppuration. Samples of sites with implants, periapical lesions, endodontic periodontal lesions, samples of patients that had taken any antimicrobial medications during the last 4 months and samples in which laboratory processing was done 48 hours after sampling were excluded.

One to four sites were sampled per patient. The sampling area was isolated with cotton rolls; the supragingival plaque was removed, and the area was air-dried. Sterile paper points were inserted into the depth of the pocket and left in place for 15 to 20 s. The points were then immediately transferred to a vial containing 2 ml of the VMGIII transport medium and sent to the laboratory.

Microbiological analysis

The samples were vortexed for 20 seconds and 10-fold serially diluted in phosphate-buffered saline

(PBS). A 100 µl aliquot of each dilution was plated on 4.3% Brucella Agar (BBL Microbiology Systems, Cockeysville, MD) containing 5% defibrinated sheep blood, 0.2% hemolyzed human red blood cells, 0.0005% hemine and 0.0005% menadione for isolation of black-pigmented *Bacteroides* and *Porphyromonas* spp. After incubation at 37°C for 10 days, the total viable count and the *P. gingivalis* count were determined. *P. gingivalis* was identified based on its typical colony morphology, small gram-negative rods on Gram stain, and positive CAAM (N- α -carbobenzoxi-L-arginine-7-aminomethylcoumarin hydrochloride) reaction on ultraviolet light fluorescence.

Association between the occurrence of *P. gingivalis* and age range

The patients were divided according to their age in 10-year intervals²⁵: age range A: 10 to 19 years; B: 20 to 29 years; C: 30 to 39 years; D: 40 to 49 years; E: 50 years or more.

Statistical Analysis

The frequency of detection of *P. gingivalis* was given as the number of positive patients for this bacterium. The chi-square test was used to assess the association between the occurrence of *P. gingivalis* and gender and age. A level of significance was set at $P < 0.05$.

RESULTS

Analysis was performed of 1,386 results of microbiological examinations of samples of subgingival microbiota of patients with periodontitis from Brazil. From these, 107 went patient original to south area of the Brasil; 1166 of the southeast area; 47 of the center-west area; and 63 of the northeast area. From these, 824 (59.4%) were of female gender and 562 (40.5%) were males. Most examinations were performed in individuals aged 40 years or more (64.3%). However, 20.4% were from patients aged 30 to 39 years, 10.7% from patients aged 20 to 29 and, 4.6% of patients aged 10 to 19 years.

The occurrence of *P. gingivalis* was observed in 17.8% of the patients, with a mean ratio of 4.71 of the total microbiota. *P. gingivalis* was present in 16% of female patients and 20.5% of male patients. The occurrences of *P. gingivalis* in the age ranges are presented in Table 1.

Table 1 – Occurrence of *P. gingivalis* according to the age range

Age range	Occurrence of <i>P. gingivalis</i>
A (10 and < 20 years)	14.1%
B (≥ 20 and < 30 years)	12.2%
C (≥ 30 and < 40 years)	20.6%
D (≥ 40 and < 50 years)	19.6%
E (≥ 50 years)	16.5%
Total	17.8%

It was observed that the occurrence of *P. gingivalis* was significantly higher in male patients ($p=0.0342$); however, there were no significant differences as to the occurrence of this bacterium at the different age ranges evaluated ($p=0.1654$) (Table 2). Based on these data, it was observed whether the occurrence of *P. gingivalis* in each age range kept its distribution, being higher among males, or if in

some age range the female gender presented a higher occurrence of this bacterium. The occurrence of *P. gingivalis* was significantly higher in the male gender for all age ranges. Calculation of the *odds ratio* revealed that the chance of presence of *P. gingivalis* in male patients with periodontitis was 1.34 times higher when compared to female patients in the same conditions (Table 3).

Table 2 – Association of occurrence of *P. gingivalis* with gender and age

Effect	Degrees of freedom	Chi-square	P value
Gender	1	4.4857	0.0342
Age range	4	6.4899	0.1654

Table 3 – Estimates of *odds ratio* for comparison of genders

Effect	Estimate	Confidence interval at 95%	
		Lower limit	Upper limit
M x F gender	1.3487	1.0226	1.7790

DISCUSSION

In the present study, *P. gingivalis* was present in only 17.8% of patients with periodontitis. This occurrence seems to be different from other populations. In individuals from Kenya with shallow periodontal pockets yet with presence of subgingival calculus, the occurrence of *P. gingivalis* was 70%¹³. Similar values were found for Romen⁵, Norwegian², Spanish²⁴, Cameroonian⁴ and

Chinese individuals²⁰ with periodontal disease. On the other hand, lower occurrences of *P. gingivalis* were found in other populations, as in patients from rural Chinese areas¹⁴, Dominican Republic²⁸, Sudan² and Netherlands²⁴. All these studies evaluated the composition of subgingival microbiota of untreated patients with periodontitis, by means of microbial culture.

Culture is considered the gold standard method for analysis of the subgingival microbiota, yet it identifies

only viable cells. As the *P. gingivalis* is a strict anaerobe, it presents greater nutritional and environmental demands to be kept viable. Thus, a prolonged time between sample collection and processing might render the cells unviable³. The aforementioned studies processed their samples in 2 to 48 hours after collection. Thus, this difference in the time period during which the specimens were kept in the transportation medium might explain the variations observed in the occurrence of *P. gingivalis*. However, some studies observed high occurrence of *P. gingivalis* even with a prolonged time of maintenance in the transportation medium. In Kenyan patients, whose samples were processed at 36 to 48 hours after collection, *P. gingivalis* was present in 70% of the patients¹³. There was also an occurrence of 75.8% in Roman individuals, whose samples were processed at 36 to 40 hours after collection⁵. In the present study, the time period between collection and processing was no longer than 48 hours. However, *P. gingivalis* was found in only 17.8% of samples. This may indicate a real variation in composition of the subgingival microbiota in patients with periodontitis from different populations.

Another aspect that might explain the variability in the occurrence of *P. gingivalis* between studies is the number of sites sampled per patient. In fact, the number of individuals positive to a certain bacterium may be higher than the number of positive sites². Thus, the highest the number of sites evaluated in each patient, the highest will be the possibility to find *P. gingivalis*.

Occurrences of 100% and 96% of *P. gingivalis* were observed in Chinese and Chilean patients, respectively evaluated by DNA probes^{19,21}. This method does not require viable cells, and identifies bacteria with higher sensitivity than the culture^{2,19,22,29}, however, it may present cross reaction with other microorganisms thus overestimating the values achieved^{4,6,19,22}. In the evaluation of the presence of *P. gingivalis* in samples of subgingival microbiota, negative sites were found for the presence of *P. gingivalis* per culture, yet positive sites were detected by DNA probes⁵. This might be explained by the absence of viable bacterial cells due to the extended period of transportation, and due to identification of these unviable cells by the DNA probes. Another possibility would be the real absence of *P. gingivalis* in the sample, leading to a negative result by culture, and cross reaction of the DNA probes would yield a false positive result. On the other hand, positive samples for the presence of *P. gingivalis* by culture and negative by DNA probes might be explained by problems in the homogenization of samples.

Other studies found high occurrence of *P. gingivalis* using the polymerase chain reaction (PCR)^{10,11,16}. *P. gingivalis* was detected more frequently in samples analyzed by PCR compared to the culture⁶. This would explain the smaller occurrence of *P. gingivalis* found in the present study, when compared to the studies using PCR. Discrepancies were also observed between PCR and DNA probes. It should be considered that the limit for detection of DNA probes is nearly 10^3 to 10^4 cells^{22,29}, whereas PCR detects microorganisms in concentrations lower than 10^2 cells⁶. Cross reactions in the analyses by DNA probes might explain the negative PCR samples and positive DNA probes^{6,22}.

Relationship has been reported between increase in age and increase in prevalence and proportion of *P. gingivalis*^{17,23,25}. Asikainen & Chen⁷ (1999) consider a relationship between presence of *P. gingivalis* and absence of *A. actinomycetemcomitans* in older individuals. *A. actinomycetemcomitans* seems to be acquired in young individuals, colonizing the incisors and first molars, which are the first permanent teeth to erupt. This localized pattern of infection would occur because of the efficiency of antibodies produced against this bacterium. On the other hand, *P. gingivalis* seems to be acquired in older individuals. The antibodies produced against this bacterium would not be able to control infection, thus a generalized pattern of distribution of *P. gingivalis* is observed in patients with periodontal disease¹⁸. However, in the present study, there was no increase in the occurrence of this microorganism with the increase in age.

Association between gender and occurrence of *P. gingivalis* in patients with periodontitis was not described in the literature. Schenkein et al.²⁶ (1993) did not find differences in the composition of subgingival microbiota between males and females.

The present study evaluated 1,386 patients with periodontitis in Brazil, which may be considered a relatively large sample when compared to other studies. The material employed for sample collection and the instructions to perform the procedure were supplied by the laboratory. This allowed standardization. However, this study presented some limitations in the evaluation of probing pocket depth (PPD). It is difficult to make comparisons according to the variations that may occur by the type of probe employed, inclination of the probe and probing force applied³⁰. For that reason, no association was performed between the occurrence of *P. gingivalis* and PPD.

Further studies are required to determine the association of *P. gingivalis* and PPD in patients with periodontitis in Brazil.

CONCLUSIONS

The occurrence of *P. gingivalis* in patients with periodontitis in Brazil was 17.8%, presenting association with gender, yet with no association with age.

RESUMO

A proposta deste estudo foi avaliar a ocorrência de *P. gingivalis* em pacientes com periodontite no Brasil e investigar se existe associação entre sua ocorrência com gênero e faixa etária. Análise foi realizada em 1386 resultados de exames microbiológicos de amostras da microbiota subgingival de pacientes com periodontite. Coleta foi realizada por periodontistas em clínicas privadas e processadas por cultura em laboratório clínico de microbiologia oral. O teste qui-quadrado foi usado para verificar a associação da ocorrência de *P. gingivalis* com gênero e faixa etária. Foi observado que 59% dos exames foram realizados em mulheres e 41% em homens, com a maioria dos indivíduos apresentando 40 anos ou mais de idade (64,3%). A ocorrência de *P. gingivalis* foi de 17,8%. Não houve diferença quanto à presença desta bactéria nas diferentes faixas etárias, mas houve ocorrência significativamente mais alta nos homens. Foi observado que, apesar da faixa etária, a ocorrência de *P. gingivalis* foi sempre maior nos homens. Foi concluído que a ocorrência de *P. gingivalis* em pacientes com periodontite no Brasil foi de 17,8% sendo associada com gênero, mas não associada com faixa etária.

UNITERMOS

Porphyromonas gingivalis; doenças periodontais; medida de ocorrência de doenças, masculino, feminino, meia-idade, análise microbiológica

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