OCCURRENCE OF *PORPHYROMONAS GINGIVALIS* AND ITS ANTIBACTERIAL SUSCEPTIBILITY TO METRONIDAZOLE AND TETRACYCLINE IN PATIENTS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Chronic periodontitis is a multifactorial infectious disease associated with Gram-negative strict anaerobes which are immersed in the subgingival biofilm. Porphyromonas gingivalis, an important periodontal pathogen, is frequently detected in patients with chronic periodontitis. Although isolates of P. gingivalis tend to be susceptible to most antimicrobial agents, relatively little information is available on its in vitro antimicrobial susceptibility. The aim of this study was to determine the frequency of P. gingivalis in patients with chronic periodontitis and to assess antimicrobial susceptibility in terms of minimum inhibitory concentration (MIC) of clinical isolates to metronidazole and tetracycline. A descriptive, observational study was performed including 87 patients with chronic periodontitis. Samples were taken from the periodontal pocket using paper points, which were placed in thioglycollate broth. Samples were incubated for 4 hours at 37°C in anaerobic conditions and finally replated on Wilkins-Chalgren anaerobic agar (Oxoid). Bacteria were identified using the RapID™ANAII system (Remel) and antimicrobial susceptibility was determined with the M.I.C. Evaluator test (MICE, Oxoid). P. gingivalis was identified in 30 of the 87 patients with chronic periodontitis, which represents a frequency of 34.5%. All 30 isolates (100%) were sensitive to metronidazole, with MIC values ranging from 0015-4ug/ml. Regarding tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug /ml, the remaining three isolates (10%) were resistant to tetracycline with MIC values of 8ug/ ml. There was no statistically significant difference in age, gender, pocket depth, clinical attachment level and severity of periodontitis between the group of patients with chronic periodontitis and P. gingivalis and the group of patients with chronic periodontitis without P. gingivalis. In conclusion, P. gingivalis was found at a frequency of 34.5% in patients with chronic periodontitis and clinical isolates were highly sensitive to metronidazole and tetracycline.

Key words: *Porphyromonas gingivalilis; Microbial Sensitivity Test; Chronic Periodontitis*

PRESENCIA Y SUSCEPTIBILIDAD ANTIBACTERIANA DE *PORPHYROMONAS GINGIVALIS* A METRONIDAZOL Y TETRACICLINA EN PACIENTES CON PERIODONTITIS CRÓNICA

RESUMEN

La periodontitis crónica es una enfermedad infecciosa multifactorial asociada a bacilos Gram-negativos anaeróbicos estrictos que se encuentran inmersos en la biopelícula subgingival. Porphyromonas gingivalis, importante patógeno periodontal, es frecuentemente detectado en pacientes con periodontitis crónica. Los aislamientos clínicos de P. gingivalis tienden a ser susceptibles a la mayoría de agentes antimicrobianos; sin embargo, se tiene poca información sobre la susceptibilidad antimicrobiana in vitro. El objetivo de este estudio fue determinar la frecuencia de P. gingivalis en pacientes con periodontitis crónica y determinar la susceptibilidad antimicrobiana en términos de concentración inhibitoria mínima (CIM) de los aislamientos clínicos a metronidazol y tetraciclina. Se realizó un estudio observacional descriptivo en el que se incluyeron 87 pacientes con periodontitis crónica. Las muestras tomadas con conos de papel de la bolsa periodontal se depositaron en caldo tioglicolato, se incubaron durante 4 horas a 37 °C en anaerobiosis y se resembraron en agar anaeróbico Wilkins-Chalgren (Oxoid). La identitficación de los aislamientos se realizó con el sistema RapID™ ANA II (Remel) y la susceptibilidad antibiótica para metronidazol y tetraciclina se evaluó mediante la técnica M.I.C.Evaluator (M.I.C.E., Oxoid). En 30 de los 87 pacientes con periodontitis crónica se identificó P. gingivalis, lo que representa una frecuencia de 34.5%. Todos los 30 aislamientos (100%) fueron sensibles al metronidazol con valores de CIM desde 0.015 hasta 4 ug/ml. En cuanto a tetraciclina, 27 aislamientos (90%) fueron sensibles con valores de CIM desde <0.015 hasta 4 ug/ml; los restantes 3 aislamientos (10%) fueron resistentes a tetraciclina con valores de CIM de 8 ug/ml. En cuanto a edad, género, profundidad de bolsa, nivel de inserción clínico y severidad de la periodontitis no se presentaron diferencias estadísticamente significativas entre el grupo de pacientes con periodontitis crónica y P. gingivalis y el grupo de pacientes con periodontitis crónica sin P. gingivalis. En conclusión, P. gingivalis se encontró en una frecuencia de 34.5% en pacientes con periodontitis crónica y los aislamientos clínicos fueron altamente sensibles a metronidazol y tetraciclina.

Palabras clave: *Porphyromonas gingivalis; test de sensibilidad microbiana; periodontitis crónica*

INTRODUCTION

Periodontal disease is an infectious oral disease which affects many people in the world[1-5]. Measured by clinical attachment loss, it affects 50.2% of the population in Colombia[6].

Periodontitis is defined as an inflammation compromising the whole tooth supporting apparatus and classified as chronic, aggressive and associated to systemic diseases[1,2]. Chronic periodontitis is the most frequent form of periodontal disease, and because of its insidious, asymptomatic behavior, is nearly always diagnosed at an advanced age and even in the terminal stages of the disease[1,2,7,8]. It leads to progressive attachment loss and bone loss and is characterized by the formation of pockets which can affect a variable number of teeth in different stages of progression[1,2,7]. Factors inherent to the host, smoking and environmental factors are important and determinant in its evolution and severity[1,2,7,8].

Chronic periodontitis is a multifactorial infectious disease and several microorganisms are involved in its etiology, among which *Porphyromonas gingivalis* is of vital importance due to its virulence factors and the role it plays in the development of periodontal pathology[1,2, 7-10].

P. gingivalis is a Gram-negative, obligate anaerobe rod, which produces black-brown colonies on anaerobic blood agar, and in the oral cavity is found mainly immersed in the subgingival microflora[1,2,7]. It meets the criteria to be considered a pathogen: it stimulates the host's immune response, evades defense mechanisms and destroys host tissues by secreting its own substances [1,10,11].

Different studies have shown that the frequency and distribution of periodontal microorganisms in the subgingival microflora, in particular *P. gingivalis,* is variable according to factors such as geographic region, race, diet, development level and living conditions, among others [1-3,9-13].

When antimicrobial therapy is needed in patients with chronic periodontitis for the eradication of *P. gingivalis,* its susceptibility or resistance profile to antibiotics needs to be known [11,14,15]. Various different susceptibility patterns have been found for *P. gingivalis*. [16-21].

In vitro antimicrobial susceptibility tests can be used to determine microorganism profiles and changes in behavior in response to different periodontal therapies, with the aim of contributing to developing adequate antibiotic management policies and delaying the appearance of antimicrobial resistance[11,20-22].

The aim of this study was to determine the frequency of *P. gingivalis* in patients with chronic periodontitis and to determine its antimicrobial susceptibility to metronidazole and tetracycline.

MATERIALS AND METHODS

Study characteristics

This was an observational, descriptive study of 87 patients diagnosed with untreated chronic periodontitis (localized chronic periodontitis and generalized chronic periodontitis) who visited the pre-graduate and post-graduate Periodontal Clinics at the School of Dentistry of Pontificia Universidad Javeriana, from May 2011 to July 2012.

Clinical study

A previously calibrated researcher performed the clinical periodontal evaluation (full mouth) on all patients using a Williams probe (Williams color-coded probe PQW, Hu-Friedy, Chicago-Illinois, USA), including gingival margin, bleeding on probing, pocket depth and clinical attachment level, and patients were classified following the recommendations of the 1999 International Consensus of the American Academy of Periodontology[23]. Periodontal probing was performed and 6 surfaces of all teeth were measured to select the one which would be included in the sample (mesial-buccal, mesial-lingual/palatal and buccal/palatal interproximal surfaces).

Inclusion criteria were: patients diagnosed with chronic periodontitis, with at least 10 teeth, without systemic compromise, over 18 years old, who had not received previous periodontal therapy (for at least 6 months). Exclusion criteria were: patients who had taken antibiotics, corticoids or non-steroid analgesics within three months prior to the sampling, pregnant or lactating women, and smokers. The study was approved by the Ethics and Research Committee at the School of Dentistry of Pontificia Universidad Javeriana. All patients signed informed consent which described the nature of the project and associated benefits. A survey was conducted to determine each patient's systemic condition, which also provided information on whether the inclusion and exclusion criteria were met.

Microbiological study

Samples were taken by selecting 5 sites with pocket depth ≥4mm and clinical attachment level ≥ 2 mm. The supragingival biofilm was removed with sterile gauze, the zone was isolated with sterile cotton, and paper points (New Stetic®) were placed in the periodontal pocket for 1 minute. The paper points were removed and placed in Eppendorf tubes with 900 ul thioglycollate broth (BBL™ Fluid, Becton Dickinson and Company) supplemented with hemin and menadione[24-27], and placed in jars with anaerobiosis generating envelopes (Anaerogen, Oxoid) until they arrived at the laboratory.

Isolation and identification of P. gingivalis

In the laboratory, the samples in the anaerobiosis jars were incubated for 4 hours at 37°C in order to enrich and thus multiply the anaerobes [26,27]. After incubation, they were centrifuged (Eppendorf® centrifuge) at 4000 rpm for 10 minutes. Of the centrifuged product, 300µl were removed and the remaining 600µl were vortexed (Maxi mix II Thermolyne®) to produce a homogeneous mixture of the sample. Then the rest of the thioglycollate broth was used to make a series of five dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) in thioglycollate broth, to isolate *P. gingivalis.* Fifty ul of the three latter dilutions only (10^{-3}, 10^{-4}, 10^{-5}) were re-plated en masse on Wilkins-Chalgren (Oxoid) anaerobic agar supplemented with 1% (v/v) hemin and menadione and 5% (v/v) lamb`s blood, and incubated at 37°C for 8 days in anaerobic atmosphere. After the incubation period, types of colony present in the culture medium were observed (black-brown pigmented and non-pigmented), Gram stained and exposed to long-wave ultraviolet light to show presence or absence of fluorescence. Absence of fluorescence is considered to be a quick test to distinguish between *P. gingivalis* and other Gram-negative anaerobe rods with black-brown pigmentation[28-30]. Colonies presumed to be *P. gingivalis* were plated again for an air tolerance test in Wilkins-Chalgren agar (Oxoid) supplemented with 1% (v/v) hemin and menadione, and 5% (v/v) lamb's blood, and incubated at 37°C for at least 8 days. Finally, the obligate anaerobic colonies were selected and their purity was confirmed by Gram stain. Pure isolates of obligate anaerobes were identified with the RapID™ ANA II system (Remel). The respective quality controls were included in order to ensure that tests were performed correctly.

Antibacterial susceptibility test

After isolation and identification of *P. gingivalis,* its antibiotic susceptibility (minimum inhibitory concentration - MIC) to metronidazole and tetracycline was evaluated using the M.I.C-Evaluator technique (M.I.C.E., Oxoid). All 30 strains of *P. gingivalis* were isolated again on anaerobic Wilkins Chalgren agar (Oxoid) supplemented with 1% (v/v) hemin and menadione and 5% (v/v) lamb's blood. All isolates were incubated at 37°C for 5 days in anaerobic atmosphere in order to produce fresh colonies for subsequent susceptibility tests, following CLSI M100-S22 standards and the recommendations for evaluating susceptibility with M.I.C-Evaluator. After 5 days' incubation, the purity of the isolates was examined again, and suspensions of each of the 30 isolates were prepared in isotonic sterile saline solution and adjusted to 1 on the Mc Farland scale. A brush was used to plate all suspensions en masse on Wilkins-Chalgren anaerobic agar (Oxoid) supplemented with 1% (v/v) hemin and y menadione, and 5% (v/v) lamb's blood, and incubated at 37°C for 5 days in anaerobic atmosphere. Finally, the MIC was read, following the manufacturer's instructions and taking into account the cut-off points for the antibiotics evaluated: values ≤ 8ug/ml for metronidazole and ≤ 4ug/ml for tetracycline were considered sensitive.

Statistical analysis

Descriptive univariate and bivariate statistical analysis was performed (distribution of frequencies of categorical variables, mean and standard deviation of continuous variables). A U-Mann Whitney test was used to determine whether there are differences between presence and absence of *P. gingivalis* according to pocket depth, level of attachment, severity of periodontitis and age. The Chi square test was used to determine differences between presence and absence of *P. gingivalis* according to the variables sex and bleeding on probing. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Table 1 shows the demographic and clinical characteristics of patients with chronic periodontitis with or without presence of *P. gingivalis*. *P. gingivalis* was identified in 30 of the 87 patients with chronic periodontitis, representing a frequency of 34.5%.

Table 1: Principal demographic and clinical findings in the 87 patients with chronic periodontitis included in the study, according to presence or absence of *P. gingivalis*.

Caracteristics		*P. gingivalis* Present	*P. gingivalis* Absent	Statistics
Number		30 (34.5%)	57 (65.5%)	
Age (years)		45.63 ± 12	46.73 ± 12	P > 0.834*
Sex	Female	15	33	P = 0.482**
	Male	15	24	
Sites with Bleeding on Probing (%) [a]		97.3 ± 14	95.9 ± 12	P > 0.672**
Pocket depth (mm) [a]		5.62 ± 1.4	5.77 ± 1.6	P > 0.6514*
Attachment level (mm) [a]		5.77 ± 3.1	5.56 ± 1.33	P > 0.8687*
Severity of periodontitis (mm) [a]		5.56 ± 2.6	5.43 ± 1.18	P > 0.7511*

[a] Values correspond to mean ± standard deviation
* U-Mann Whitney test, **Chi squared test

Of the 87 study patients diagnosed with chronic periodontitis, 48 (55.2%) were female and 39 (44.8%) were male; and regarding age, 10 (11.5%) were 18-30 years old, 42 (48.3%) were 31-50 years old and 35 (40.2%) were 51-70 years old.

Age (mean ± standard deviation) of patients with and without *P. gingivalis* respectively, was 45.63±12 and 46.73±12 years, with no statistically significant difference (P > 0.834, Table 1). The 30 *P. gingivalis* isolates were distributed as follows: 15 isolates (50%) from the 18-30 year range, 11 (36.7%) from the 31-50 year range, and 4 (13.3%) from the 51-70 year range. There was no statistically significant difference regarding sex for patients with or without presence of *P. gingivalis* (P>0.05, Table 1).

With regard to clinical parameters, the values (mean ± standard deviation) for pocket depth in patients with and without *P. gingivalis* were, respectively, 5.62± 1.4 mm and 5.77±1.6 mm, with no statistically significant difference (P > 0.6514, Table 1). Among the 30 patients with chronic periodontitis and presence of *P. gingivalis*, pocket depth was 4-5 mm in 13 patients (43.3%), 5-7 mm in 12 (40%) and greater than 7 mm in 5 (16.7%). Loss of attachment level (mean ± standard deviation) was 5.77 ± 3.1 in patients with *P. gingivalis,* and 5.56±2.6mm in patients without *P. gingivalis*. The differences were not statistically significant (P > 0.8687, Table 1). Severity of the periodontitis did not differ significantly (P > 0.7511) between patients with *P. gingivalis* (5.56 ± 1.33) and without *P. gingivalis* (5.43 ± 1.18) (Table 1).

Percentages of sites with bleeding on probing in patients with chronic periodontitis with and without *P. gingivalis*, respectively, were (mean± standard deviation) 97.3±14 and 95.9±12, with P > 0.672 by Chi squared test, showing that the differences are not statistically significant (Table 1).

With relation to the extension of periodontal destruction, there was localized chronic periodontitis in 71.3% of patients. Of the 87 patients (48 female and 39 male) with chronic periodontitis, 40 (46%) had moderate chronic periodontitis and 47 (54%) had severe chronic periodontitis. Of the 48 females in the study, 26 had moderate chronic periodontitis and 22 had severe chronic periodontitis. Of the 39 males, 14 had moderate chronic periodontitis and 25 had severe chronic periodontitis. Of the 30 patients with chronic periodontitis in whom *P. gingivalis* was found, 14 had moderate chronic periodontitis and 16 had severe chronic periodontitis; and of the 57 patients with chronic periodontitis in whom *P. gingivalis* was not found, 26 had moderate chronic periodontitis and 31 had severe chronic periodontitis. The U-Mann Whitney statistical analysis showed no statistically significant difference between these two groups (P >0.5063).

Table 2 shows the MIC results for the 30 *P. gingivalis* clinical isolates to metronidazole and tetracycline. The strains showed widely differing susceptibility two the two antimicrobial agents. All 30 isolates (100%) were sensitive to metronidazole with MIC values ranging from 0.015 to 4 ug/ml; the highest frequency to sensitivity (n=3)

was at MIC values of 0.015, 0.03 and 0.06 ug/ml. For tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug/ml; the highest frequency to sensitivity (n=3) was at MIC values of 0.015, 0.03 and 0.06 ug/ml. The remaining 3 isolates (10%) were resistant to tetracycline, with MIC values of 8 ug/ml.

Table 2: Values for antimicrobial susceptibility to metronidazole and tetracycline at minimum inhibitory concentration for the 30 *P. gingivalis* isolates, found using the M.I.C.Evaluator system (MICE, Oxoid).

Isolates	Metronidazole ug/ml	Tetracycline ug/ml
P. gingivalis (n=3)	0.015	0.015
P. gingivalis (n=2)	0.015	4
P. gingivalis (n=3)	0.03	0.06
P. gingivalis (n=2)	0.03	< 0.015
P. gingivalis (n=1)	0.03	0.12
P. gingivalis (n=2)	0.03	2
P. gingivalis (n=3)	0.06	0.03
P. gingivalis (n=1)	0.06	0.06
P. gingivalis (n=1)	0.06	0.12
P. gingivalis (n=1)	0.12	4
P. gingivalis (n=2)	0.15	2
P. gingivalis (n=2)	0.25	0.06
P. gingivalis (n=2)	0.3	< 0.015
P. gingivalis (n=2)	0.15	8
P. gingivalis (n=1)	2	0.25
P. gingivalis (n=1)	4	0.12
P. gingivalis (n=1)	0.12	8

Values ≤ 8ug/ml for metronidazole and ≤ 4ug/ml for tetracycline are considered sensitive.

DISCUSSION

Periodontitis is considered to be a mixed infectious bacterial disease caused mainly by Gram negative anaerobes[9] which interact with host tissues and cells causing the release of a wide range of cytokines, chemokines and inflammatory mediators, leading to the destruction of periodontal structures[31].

P. gingivalis is a rod-shaped, Gram negative, immobile, asaccharolytic, strict anaerobe[9]. Because of its ability to produce a large quantity of virulence factors, it is considered to be a major pathogen and very important microbiological indicator in the onset and development of periodontal disease[31].

This study reports the frequency and antimicrobial susceptibility of *P. gingivalis* isolated from patients with chronic periodontitis. *P. gingivalis* was found with a frequency of 34.5% (30/87) and all 30 isolates were highly sensitive to metronidazole (100%-30/30) and tetracycline (90%-27/30). This frequency was lower than those reported since 2007 in patients with chronic periodontitis in Iran (41.7%)[20], Spain (77.8%)[2], Japan (78.5%)[32] and Chile (83.8%)[2]. Previous studies in Colombia report frequencies of *P. gingivales* in patients with chronic periodontitis of 60.7%, 65.9%, 67.1%, 68.2 and 76.47% [1-4,33]. It would seem that these variations in frequency are a result of differences in sample taking, transportation and processing, isolation by bacteriological culture and the use of molecular techniques, and particular socio-cultural, demographic and living conditions [1,3-5,17,20,32,33]. The study by Sanai et al.[17] clearly shows that the transportation of samples could have caused loss of viability in *P. gingivalis, P. intermedia* and *P. nigrescens.* The differences in sensitivity obtained by culture may also be due to situations generating changes in the subgingival microflora, including deficient hygiene habits and attitudes, chronic baseline diseases, smoking, alcohol use and previous antimicrobial therapies [2-4,11,23].

Thioglycollate broth is an enrichment medium designed to facilitate rapid growth of a wide variety of fastidious, aerobic, microaerophilic microorganisms, and in particular obligate anaerobes[24-27]. This study used thioglycollate enriched with hemin and menadione, containing a base of casein, L-cystine, dextrose, yeast extract, sodium chloride, sodium thioglycollate, resazurin and a low proportion of agar to provide a soft consistency[24-27]. L-cystine and sodium thioglycollate are reducing agents that maintain a low oxidation-reduction potential which allows survival and adequate metabolism of obligate anaerobes. Hemin and menadione stimulate the multiplication of bacteria that produce black-brown pigment, among which *P. gingivalis* is included[26,27].

According to scientific and technical principles (DNA amplification), molecular techniques (in particular PCR –polymerase chain reaction) are assumed to be more sensitive and specific than the culture method [34,35]. Studies conducted using cultures on patients with chronic periodontitis have found some sensitivities which are low, while others are very close to or even higher than (41.7, 60.7,

67.1, 65.9, 76.47, 77.8 and 83.8%)[1,2,4,20,33] those reported in studies using the PCR technique (68.2 and 78.5%)[3,32]. It should be noted that these sensitivities were found in different populations and social-demographic situations, in addition to which they were conducted on different sample sizes. There is currently only one paper which attempts to resolve the inconsistencies in sensitivity between the two methods[35]. The study by Urban et al.[35] detects periodontal pathogenic bacteria using the traditional anaerobic culture method and commercial PCR. The PCR test detected almost the same number of positive samples for *P. gingivalis* as the culture method[35], with 94% concordance and only two discrepant results. From these results it can be deduced that commercial PCR can be recommended for use in an oral microbiological diagnosis laboratory for its speed (2-3 hours), sensitivity and specificity. However, even though the culture method is tedious, slow and requires expertise, it allows antimicrobial susceptibility to be evaluated and enables other studies that require the live bacteria to be used in typing or studies of virulence and pathogenicity. Upon selecting a method, laboratories should assess their needs, the impact the method may have on diagnosis and its limitations. The results of culture and PCR seem to indicate that joint use of both methods may be required due to the individual contributions of each[5, 35,36].

Metronidazole is a synthetic, primarily bactericidal chemotherapeutic agent. Its antibacterial action is limited to a wide range of anaerobic bacteria[14]. It is often used in the treatment of severe periodontitis, and is frequently the medication of choice and used empirically in combination with one or more other antimicrobial agents[14]. Tetracyclines are a family of large structures, natural or semi-synthetic antibiotics, and basically bacteriostatic agents which act by stopping protein synthesis[14].

The M.I.C.Evaluator system used in this study has been perfectly proven and provides fast, reliable results for determining MICs, as it does not require dilutions of the antimicrobial agent and it avoids the excessive use of culture mediums[37]. All 30 *P. gingivalis* isolates were sensitive to metronidazole with MIC values ranging from 0.015 to 4 ug/ml. For tetracycline, 27 isolates (90%) were sensitive, with MIC values ranging from <0.015 to 4 ug/ml, with the remaining 3 isolates (10%) being resistant to tetracycline with MIC values of 8 ug/ml. Similarly, the study by Andrés et al.[16] in 1998 reports that 100% of 31 *P. gingivalis* were susceptible to metronidazole and tetracycline, with MIC <0.125-2 ug/ml for metronidazole and <0.125.0.5 ug/ml for tetracycline. Kulik et al.[21] evaluated the antimicrobial susceptibility of 152 *P. gingivalis* strains to metronidazole and tetracycline, among other antimicrobial agents. All isolates were 100% susceptible to both these antimicrobial agents, with MIC <0.016-0.016 ug/ml for metronidazole and <0.016-2 ug/ml for tetracycline. Japoni et al.[20] reported 100 and 94% susceptibility, respectively, to doxycycline and metronidazole of 50 *P. gingivalis* strains isolated from patients with chronic periodontitis in Iran. Van Winkelhoff et al.[38] report susceptibilities of 100% to metronidazole and tetracycline in clinical isolates of *P. gingivalis* from Holland and Spain. In contrast to these high susceptibilities, Ardila et al.[19] report 21.56 % (11/51) resistance of *P. gingivalis* to metronidazole with MIC values of 0.08-16 ug/ml. With the exception of this high resistance to metronidazole, all other studies report high sensitivity to it. High resistance to metronidazole and other antimicrobial agents may be due to the excessive and inadequate use of antimicrobial agents, which foster the development of highly resistant strains[19,]. Situations leading to bacterial resistance should be avoided in day to day practice[14,15].

In our study, the 10% resistance to tetracycline is noteworthy. The most common resistance mechanism to tetracycline is by protein synthesis of the efflux pumps, which in Gram negative microorganisms are encoded by the *tet* genes[14]. Sanai et al.[17] (2002) determined the presence of the gene providing resistance to tetracycline (tet-Q) in 3 out of 5 (60%) *P. gingivalis* isolates from children, and these isolates seem to belong to the same original clone. It is important to consider that bacteria which are resistant to antimicrobial agents and live in the oral cavity may be an important source of transmission of genes providing antimicrobial resistance to other pathogenic bacteria. In the near future, the search for these resistant genes should probably look at the 3 tetracycline-resistant *P. gingivalis* strains reported herein.

The aim of this study was to determine whether gender and age were related in any way to the presence or absence of *P. gingivalis* in patients with chronic periodontitis. No relationship was found. These

results agree with those reported in other studies conducted in Colombia[1,4].

Different studies show that there is a very high association between *P. gingivalis* and signs of periodontal disease: inflammation, increase in probing depth, poor oral hygiene, loss of alveolar bone, loss of clinical attachment, bleeding on probing and severity of periodontitis[22,31]. In addition, the presence of *P. gingivalis* seems to play a very important part in the progression of chronic periodontitis[22,24]. Nevertheless, this study found no relationship between pocket depth, bleeding on probing, attachment level or severity of periodontitis and presence or absence of *P. gingivalis,* in agreement with the results reported by Lafaurie et al.,[3] and in contrast to other studies which found that *P. gingivalis* is strongly related to pocket depths > 5mm[3,9,39].

The results presented in this study are a contribution to the microbiological study of chronic periodontitis in the population of Colombia. They should contribute to devising preventive measures and developing adequate policies for managing antibiotics, to delay the appearance of antimicrobial resistance.

To conclude, this study reports a *P. gingivalis* frequency of 34.5% in patients with chronic periodontitis, and the clinical isolates identified had high sensitivity to metronidazole and tetracycline.

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