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Antibacterial susceptibility patterns of *Porphyromonas gingivalis* isolated from chronic periodontitis patients

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Abstract

Objectives: To test the antimicrobial sensitivity of *Porphyromonas gingivalis* to a panel of eight orally administrable antibiotics in chronic periodontal diseases and to evaluate factors associated with periodontitis in adult patients.

Study Design: A total of fifty strains of *P. gingivalis* were isolated from one hundred and twenty adult patients with chronic periodontitis. Identification of bacteria was carried out by anaerobic culture and biochemical tests. Selected colonies of *P. gingivalis* were used to evaluate the antibacterial activities of penicillin, metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin, doxy-cycline, ciprofloxacin and azithromycin.

Results: Most of the patients were female, age ranging between 40 to 50 years. Majority of the patients frequently had scaling and depths of periodontal pockets in infected teeth were 5-8 mm and most of them had hemorrhage during sampling. Susceptibility testing revealed a sensitivity of 100% of *P. gingivalis* to azithromycin, doxycycline and amoxicillin/clavulanic acid but lower susceptibilities were found for the rest of antibiotic agents evaluated.

Conclusions: Frequent scaling in women aged between 40-50 years had positive correlation with chronic periodontitis. The application of antibiotics in conjunction with mechanical debridement, may reflect in the level of resistance of *P. gingivalis* in patients with chronic periodontal infections. This could suggest periodical antibiotic susceptibility testing is necessary to determine the efficacy of antimicrobial agents if the perfect curing of chronic periodontal diseases after mechanical debridement is meant. Further clinical studies are required to confirm the in vitro results. The only limitation in this study was identification of bacteria to species rather than subspecies level.

Key words: Scaling, antibiotics, chronic periodontal diseases, resistance.

Introduction

Porphyromonas gingivalis belongs to the genus *Bacteroides* and is a non-motile, gram-negative, rod-shaped, anaerobic pathogenic bacterium. It forms black colonies on blood agar. *P. gingivalis* is pathogenic bacterium, commonly found in the human body and especially in the oral cavity, associated with periodontal lesions, infections, and adult periodontal diseases (1).

The American Academy of Periodontology classification system was established to identify the distinct types of periodontal diseases by taking into consideration factors such as age of onset, clinical appearance, and rate of disease progression, pathogenic microbial flora and systemic influences (2). Periodontitis, a bacterially induced, localized, chronic inflammatory disease, destroys connective tissues and bones that support the teeth. It is common, with mild to moderate forms affecting 30% to 50% of the adults and the severe, chronic forms affecting 5% to 15% of all adults in the United States (3). Periodontitis has even higher prevalence in developing countries and considerable global variation, although the prevalence of the severe generalized disease appears to be similar in most populations (4).

Drug resistance of *P. gingivalis* in periodontal diseases is implicated in the pathogenesis of periodontitis (5,6). These bacteria are able to produce virulence factors that act locally within the sulcus, and result in tissue destruction (5,6). Among them, cell-surface-associated and secreted proteinases such as Arg-gingipain and Lys-gingipain and proteolytic enzymes have received much attention because they can degrade various host proteins and cause inflammation (6). *P. gingivalis* has shown the ability to invade human gingival fibroblasts and oral epithelial cells in cell culture (7). It has been reported that from 13 isolates recovered from protected bronchoalveolar lavage fluid, nine respiratory pathogens matched genetically those recovered from the corresponding dental plaque of eight patients (8). Based on the recent reports, systemic antibiotics should not be used in most adult patients with periodontitis. Therefore, their application should be restricted to specific conditions such as aggressive or severe progressive periodontitis (9). Clindamycin, metronidazole and amoxicillin are the antibiotics commonly used in conjunction with mechanical debridement (10-12). Besides, positive responses have been reported with amoxicillin/clavulanic acid in the treatment of periodontitis (10,12).

The present study aimed to test, *in vitro*, the susceptibility of *P. gingivalis*, isolated from periodontal pockets in adult patients with chronic periodontitis, to eight orally administrable antibiotics and to investigate the factors associated with chronic periodontal disease trigger.

Materials and Methods

Selection of patients

Inclusion criteria

One hundred twenty adults with chronic periodontal

disease, attending the clinic of dentistry, division of periodontology, Shiraz, Iran, from June 2008 to March 2009 were enrolled in the study. Written informed consents were obtained from all the patients, and approved by the ethics board of Shiraz University of Medical Sciences. Patients with generalized to severe, chronic periodontitis with more than 3mm attachment loss in more than 30% of teeth and with 4-7 mm true periodontal pockets, were included in the study (13).

Exclusion criteria

Patients with the following conditions were excluded from the study: pregnancy, systemic diseases that influence periodontium or interfere with examination or sampling, smoking, consuming antibiotics during last three months, corticosteroid consumption and history of periodontal disease during last six months.

Sampling

One hundred and twenty patients with the above-mentioned characteristics were selected. One tooth from each patient was selected (120 teeth), after isolation of the tooth with cotton roll, supragingival was removed with curette and gingival crevicular fluid was collected by insertion of two paper points into pocket for 30 seconds. Paper points were then transferred quickly into 2 ml transport media (brucella broth; BBL Microbiology Systems, Cockeysville, Md. supplemented with 0.4- μ l/ml vitamin K1 and 5- μ g/ml hemin; Sigma Chemical Co.).

Isolation of P.gingivalis

Brucella broth containing patients' samples were then diluted and plated out onto trypticase soy agar (BBL Microbiology Systems), supplemented with 10% defibrinated horse blood, 5- μ g/ml hemin, and 0.4- μ l/ml vitamin K1. The plates were incubated in duplicate in an anaerobic atmosphere containing 80% N₂, 10% CO₂ and 10% H₂ for 7 to 10 days. The bacteria were selected on the basis of size, color, shape, staining and biochemical tests. Those with black-pigmented colonies, gram-negative rods were submitted to a fluorescence test by long wave UV light. Absence of fluorescence was considered as a rapid taxonomic test to distinguish between *P. gingivalis* and other black-pigmented, anaerobic, gram-negative rods (14). Isolates were then identified as *P. gingivalis* based on catalase and indole tests, glucose fermentation and sheep red blood cell agglutination. In each of the anaerobic culture batch, standard type strain (American Typing Collection; ATCC 33277) was also included to facilitate the comparison of bacterial colonies, morphology, and results of biochemical tests of the isolated bacteria with the standard type.

Antibacterial susceptibility patterns

The minimal inhibitory concentrations (MICs) of fifty isolates of *P. gingivalis* to eight antibiotics including, metronidazole, penicillin, clindamycin, ciprofloxacin, doxycycline, amoxicillin, amoxicillin/clavulanic acid and azithromycin were determined by the E test method (AB Biodisk, Sweden). American Typing Collection (ATCC

33277) of *P. gingivalis* was used as a control strain in antibacterial susceptibility testing. Bacterial strains were grown on blood agar plates (Oxoid no. 2, Basingstoke, UK), supplemented with 5% sheep blood, hemin (5 mg/l) and 0.4-µl/ml vitamin K1 for 5 days. Then, test organisms were suspended in sterile phosphate-buffered saline equivalent to a 0.5 McFarland standard and streaked confluenty over the surface blood agar plates. Plates were incubated in 80% N₂, 10% CO₂ and 10% H₂ for 3 to 5 days. Inhibition zones were measured according to the recommendations of the manufacturer.

Results

Of one hundred twenty patients enrolled in the study, *P. gingivalis* isolated from 50 (41.7%) of the them. Most pa-

tients were female (62% versus 38%) and the age range of 40 to 50 years was predominant (54%), compared lower or upper age ranges, (24% or 22%, respectively) (Table 1). As for the educational status of the patients, the majority of them were undergraduate. As revealed, most of them had daily brushing (72%) and never used dental floss (96 %) and they had several times scaling (72%). In 62 % of the patients, depths of periodontal pocket were 5-8 mm and majority of them (96%) had hemorrhage during sampling (Table 1). Based on antibacterial susceptibility determination, three antibiotics had perfect (100%) activities against *P. gingivalis* while the rest showed 60 to 96% efficacy against the isolates (Table 2). MIC₅₀ and MIC₉₀ of the three most effective antibiotics were lower, compared with the rest of

Table 1. Demographic information and factors associated with chronic periodontitis in 50 adult patients.

Demographic data and factors associated with periodontitis		Number	Percentage
Gender	Male	19	38
	Female	31	62
Age	30-40	12	24
	40-50	27	54
	>50	11	22
Education	Under graduate	31	62
	Graduate	19	38
Brushing	Seldom	7	14
	Several times a week	7	14
	Daily	36	72
Use of dental floss	Daily	2	4
	Never	48	96
Scaling	Several times	36	72
	Never	14	28
Depth of periodontal pocket	5-8 mm	31	62
	>8 mm	19	38
Hemorrhage when sampling	Observed	48	96
	Not observed	2	4

Table 2. Minimum inhibitory concentration (MIC) of fifty strains of *P. gingivalis* isolated from the patients with chronic periodontal diseases.

Antibiotic	MIC range(µg/ml)	MIC ₅₀ ^a (µg/ml)	MIC ₉₀ ^a (µg/ml)	Sensitive n (%)	Intermediate resistant n (%)	Resistant n (%)
Metronidazole	0.016-≥1	0.016	0.5	47 (94)	0	3 (6)
Penicillin	0.002-1	0.023	0.5	46 (92)	1 (2)	3 (6)
Co-Amoxiclav	0.016-0.125	0.016	0.125	50 (100)	0	0
Clindomycin	0.016-0.08	0.016	0.047	48 (96)	0	2 (4)
Doxycycline	0.016-0.5	0.032	0.5	50 (100)	0	0
Amoxicillin	0.016-2	0.024	1	44 (88)	2 (4)	4 (8)
Ciprofloxacin	0.002-1	0.094	0.75	30 (60)	16 (32)	4 (8)
Azithromycin	0.002-0.38	0.032	0.38	50 (100)	0	0

^a MIC50 and MIC90 indicate the MIC values at which 50% and 90% of the tested isolates were inhibited, respectively.

Table 3. Cross-resistance of fifty isolates of *P. gingivalis* to the tested antibiotics.

Number of isolates and percent (value in parenthesis) resistant to						
Number		MET	PEN	CLN	AMC	CIP
MET	3		0	1(33.3)	1(33.3)	1(33.3)
PEN	3	0		1(33.3)	1(33.3)	1(33.3)
CLN	2	1 (50)	1 (50)		0	2 (100)
AMC	4	1 (25)	1 (25)	0		2 (100)
CIP	4	1 (25)	1 (25)	2 (50)	0	

Abbreviations: MET; metronidazole, PEN; penicillin, CLN; clindamycin, AMC; amoxicillin, CIP; ciprofloxacin.

the antibiotics. Antibiotic resistant isolates expressed cross-resistance to the other tested antibiotics, ranging between 33.3% to 100% (Table 3).

Discussion

P. gingivalis is considered as a major etiological agent in the onset and progression of chronic destructive periodontitis. In the present study, associations between several factors with chronic periodontitis have been observed. Majority of the patients were women (62%) aging between 40-50 years old. The greater incidence of periodontitis among the females may be due to inappropriate attention to the oral hygiene. Hormonal production and experiences of the pregnancies in females within the age range of 40-50 years could cause reduction in the composition of body minerals including bone and teeth (15,16), which may predispose them to more periodontal diseases (17). It has also been proposed that high education could reflect in quality of life, so that the undergraduate patients in this study may not have had enough attention to their oral hygiene (18). Majority of the patients in this investigation had daily brushing but as dentists' reports inadequate or wrong brushing is also evident. This might be the reason why periodontal disease was even more common among those with regular brushing (19). In this study the majority (72%) of the patients previously had several times scaling. The need for scaling may also be associated with poor oral hygiene and smoking (20,21). Therefore, it could be direct association between periodontal diseases and dental caries with scaling specially when carried out frequently in short period of time. It has been suggested that depths of periodontal pocket in the patients with aggressive infections are deeper when compared with patients with moderate periodontitis (22), as also seen in the present study. Nevertheless, in addition to the factors mentioned above, other factors such as smoking and special food

habits may be associated with periodontal disease and should be taken into account (23,24).

The results of the present study showed that three antibiotics including amoxicillin/clavulanic acid, doxycycline and azithromycin had 100% activities against *P. gingivalis* which can provide alternative antibiotics to treat the patients whenever indicated. The fact that 12% of the bacteria were resistant or intermediate resistant to amoxicillin but 100% were sensitive to amoxicillin/clavulanic acid, indicates that resistance of *P. gingivalis* to β -lactam antibiotics are due to β -lactamase production (25,26). Low values of MIC₅₀ and MIC₉₀ of the three effective antibiotics were also noticed in this study, which could suggest that systemic antibiotic therapy may deliver adequate drug inside the infected teeth (27,28). However, this speculation needs to be confirmed with clinical trials. Availability of alternative effective antibiotics could help the clinicians appropriately prescribe them based on the patients' individual conditions. Resistance to the five antibiotics, as observed in our study, implies the rational use of effective antibiotics to prevent the increasing resistance rate and to preserve the efficacy of the antibiotics. In agreement with the present study, temporal and geographic changes in antibiotic susceptibility among anaerobes have been reported in Europe and Latin America (29,30). The only limitation in this study was identification of bacteria to species rather than subspecies level. Further studies into the identification of bacteria at subspecies level, using API 20A method are recommended.

In summary, the present study reveals a positive correlation of gender, age and scaling with chronic periodontitis in adults. Three antibiotics including azithromycin, doxycycline and amoxicillin/clavulanic acid were found to be 100% active against *P. gingivalis* in vitro, however, this finding needs to be confirmed with further clinical trials.

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Conflict of Interest and Source of Funding Statement

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