

Metronidazol susceptibility testing of porphyromonas gingivalis associated with adult periodontitis (An invitro study)

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ABSTRACT

Purpose: A variety of systemic agents being able to arrest the progression of periodontal disease have been studied for progression in periodontal therapy. Metronidazole usually used as a systemic and local antibiotic for treating periodontitis in Iran. During recent years an increasing number in antibiotic resistance has been documented. The purpose of the present study was to evaluate the minimal inhibitory concentration of metronidazole against clinical isolates of Porphyromonas gingivalis from adult periodontitis in Iran.

Materials & Methods: This experimental study was performed on samples of 62 patients. The samples were taken by sterile paper points. Isolated bacteria were determined by conventional methods and MIC (minimal inhibitory concentration) determination was carried out according to the agar dilution method recommended for anaerobic bacteria by NCCLS (M11-A5). The range of final antibiotic concentration was 0.0625µg/ml to 16µg/ml. The results were analyzed by interval estimates.

Results: Thirty six strains of P. gingivalis were isolated from 62 patients. 16.7%, 55.5% and 27.8% of isolated strains were susceptible to the metronidazole with final concentration of 1, 0.5 and 0.25µg/ml of metronidazole, respectively. Therefore, all strains were susceptible to metronidazole.

Conclusion: This is the first report to testing the sensitivity of P. gingivalis to Metronidazole in Iran. Fortunately, all 36 isolated strains of P. gingivalis were sensitive to metronidazole and this antibiotic may be used for treating P. gingivalis related infection in Iran.

Keywords: Porphyromonas gingivalis, Adult Periodontitis, Metronidazole.

INTRODUCTION

Porphyromonas gingivalis (P. gingivalis) is one of the most strongly active organisms associated with adult periodontitis and also seems to play a role in the pathogenesis of the other forms of periodontitis.⁽¹⁾ There are not many studies in the dental literature that are dealing with the problem of antibiotic resistance in periodontal microflora. In recent years an increasing number of antibiotic resistances have been documented for periodontal pathogens. 20 to 30% of sub-

gingival microorganisms isolated from patients with adult periodontitis are said to be resistant to Tetracycline and 15 to 30% to the Penicillin.⁽²⁾ Also in studies conducted by Sutter et al, Pascale et al, Abu-Fanas et al and Olsvik similar results were reported.⁽³⁻⁶⁾

Therefore, the question arises as to whether bacterial resistance constitutes also a major problem in the chemotherapeutic management of periodontal disease.⁽⁷⁾ The periodontal pathogens were considered to be susceptible, if the concentration of the antibiotic agent actually achievable in

gingival crevicular fluid following recommended dosage was higher than the minimal inhibitory concentration measured in vitro. One of the most frequently investigated antibiotics is Metronidazole which it is mainly active against anaerobes.⁽⁸⁻¹⁰⁾ The purpose of the present study was to evaluate the minimal inhibitory concentration (MIC) of Metronidazole against *P. gingivalis* isolated from adult periodontitis patients.

MATERIALS & METHODS

In an experimental study, a total 62 subjects were selected from the department of Periodontics, Faculty of Dentistry, Tehran University of Medical Sciences, during June to December 2002. The selected subjects were generally in good health; individuals with diabetes, autoimmune disorders, or other disorders were excluded. All subjects had adult periodontitis, based on the criteria of a minimum age of 30 years and the presence of periodontal lesions with probing depths of ≥ 5 mm. All of subjects were untreated periodontally, and none had received scaling or root planning within 2 months prior to participation in the study.⁽¹¹⁾

Microbiological sampling was carried out after careful debridement of the supragingival plaque by sterile cotton swab, but before probing. Subgingival plaque samples were taken by inserting sterile paper points to the bottom of the periodontal pocket for 10 seconds. Subgingival plaque samples were immediately placed in a vial of sterile reduced transport fluid and transported to the anaerobic laboratory.⁽¹²⁾ The samples were plated onto supplemented Brucella agar. Inoculated plates were placed in anaerobic condition created by Anaerocult A and anaerobic jar (Merck, Germany) and incubated in 35°C for 48h. After 48h incubation, the black pigmented colonies were subcultured for achieving of pure culture and identification. Related colonies were determined by conventional biochemical test.⁽¹³⁾ The MIC of Metronidazole was determined for all isolates according to the agar dilution technique

recommended for anaerobic bacteria by NCCLS.⁽¹⁴⁾ Metronidazole with final concentrations of 0.0625 μ g/ml to 16 μ g/ml were prepared by serial dilution in supplemented Brucella agar with sheep blood, Hemin and vitamin K1. Supplemented Brucella agar without antibiotic was used as control. All MIC plates were placed in the anaerobic jar and incubated in 35°C for 48hr. The MIC endpoint at the concentration where a marked reduction occurred in the appearance of growth on the test plate as compared to that of growth on the anaerobic control plates. MIC $\geq 8\mu$ g/ml was defined as susceptible.⁽¹⁴⁾ The results were listed by interval estimates.

RESULTS

Thirty-six strains of *P. gingivalis* were isolated from 62 patients. Metronidazole MIC obtained for isolated strains are presented in table1. The MIC for 10 isolates (27.8%) was 0.25 μ g/ml, for 20 isolates (55.5%) was 0.5 μ g/ml and for 6 isolates (16.7%) was 1 μ g/ml. Therefore, all of isolated strains were susceptible to Metronidazole invitro. The mean of the MIC for isolated strains was 0.514 μ g/ml.

Table1. Metronidazole MIC obtained for *P. gingivalis* isolated from adult periodontitis.

	MIC (μ g/ml)			Mean (μ g/ml)
	0.25	0.5	1	
Number of strains(%)	10(27.8)	20(55.5)	6(16.7)	0.514
Susceptibility	S	S	S	

S=Susceptible

DISCUSSION

During recent years an increasing number of antibiotic resistances in anaerobic bacteria have been documented for a vast number of microorganisms from various diseases and sources. Thus, the question arises as to whether bacterial resistance constitutes also a major problem in the chemotherapeutic management of periodontal disease. In one

study, the susceptibility of more than 300 bacterial species collected from patients with the diagnosis of adult periodontitis to 7 different antibiotics were determined.⁽²⁾ 14 to 36% of the bacterial species from these adult periodontitis patients were resistant or not susceptible to one or more of the 7 tested antibiotics.

The results seem to show a good efficacy of Metronidazole against *P. gingivalis* in vitro, agreeing with those of other study,⁽³⁾ but the mean MIC value found higher than those previous published. For example, the MIC of Metronidazole for *P. gingivalis* was reported between 0.002-0.5µg/ml.⁽¹⁵⁾ In another report, the mean of MIC of Metronidazole for *P. gingivalis* was 0.122µg/ml.⁽¹⁶⁾ According to our results, the mean of MIC in this study was 0.514µg/ml, which is higher from others.

The present investigation confirms the excellent invitro activity of Metronidazole against *P. gingivalis*. Nevertheless, the sensitivity of *P. gingivalis* is maybe decreasing against Metronidazole. Furthermore, the conventional methods of susceptibility testing seem to be necessary in these cases for monitoring the resistance.

CONCLUSION

While antibiotic resistance among anaerobes continues to increase, the frequency of antimicrobial susceptibility testing for anaerobe is declining in the world. Because anaerobic infections are often mixed and detailed bacteriology of the organisms involved may take some time, physicians must institute empiric therapy before susceptibility testing results are available. Also, economic realities and prudent use of resources mandate that careful consideration be given to the necessity for routine susceptibility testing of anaerobic bacteria. Determination of appropriate therapy can be based on published antibiograms; however, since patterns may vary within geographic regions and even within laboratories, it is strongly recommended that each laboratory periodically tests their isolates to determine

local patterns and detect any pockets of resistance.

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