

The Effect of Scaling and Root Planing on the Clinical and Microbiological Parameters of Periodontal Diseases

Marija Ivić-Kardum¹
Igor Jurak²
Koraljka Gall-Trošelj²
Krešimir Pavelić²
Andrej Aurer¹
Lejla Ibrahimagić³

¹Department of Periodontology
School of Dental Medicine
University of Zagreb
²Division of Molecular Medicine "Ruđer Bošković"
Institute Zagreb

Summary

The occurrence of periodontal pathogens in subgingival flora in periodontitis is a risk for periodontal disease progression. Therefore microbiologic diagnostic procedures are justifiably indicated in the detection of pathogens, monitoring of therapy success and outcome of the disease. The aim of this study was to show the effect of scaling and root planing on clinical and microbiological factors in 28 patients with chronic and aggressive periodontitis. Clinical assessment and microbiological testing were performed prior to, and three months after mechanical therapy. The presence or absence of bacterial plaque, gingival bleeding, pocket depth and attachment loss were assessed before and three months after scaling and root planing. Samples of subgingival plaque taken from periodontal pockets, were analysed by polymerase chain reaction technique for the presence of seven bacterial pathogens.

Results of clinical parameters and bacterial prevalence were analysed before and after therapy by Wilcoxon Rank test.

The mean pocket depth significantly decreased from 3.9 to 3.0 mm. Clinical attachment level decreased moderately from 4.1 to 3.8 mm. Mean plaque and gingival bleeding values also decreased after therapy. The prevalence of subgingival pathogens in relation to subjects was as follows: only one pathogenic species was found in 28.6%, two were found in 46.4% and three in 14.3% of subjects. The most prevalent pathogens were bacteroides forsythus in 85.7%, Porphyromonas gingivalis in 32.1%, Actinobacillus actinomycetemcomitans and Fusobacterium in 32.1% of subjects. After therapy the prevalence of pathogens decreased moderately. The total number of tested pathogens decreased in 12 subjects and this result was statistically significant. ($p=0.001$). In 16 subjects the number of pathogens was the same, and did not increase in any of the subjects. The results indicate that the effect of scaling and root planing in the treatment of periodontitis was effective in achieving clinical and microbiological improvement by decreasing the prevalence of pathogens responsible for disease progression.

Key words: *periodontitis, scaling and root planing, microbiology*

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Address for correspondence:

Marija Ivić-Kardum
Department of Periodontology
School of Dental Medicine
University of Zagreb
Gundulićeva 5, 10000 Zagreb
Croatia

Introduction

Scaling and root planing (SRP) is one of the most commonly utilized procedures for the treatment of periodontal diseases and has been used as the “gold” standard therapy in comparison to other therapeutical procedures (1,2). It is not only used in the initial phase of the therapy but also to maintain the conditions achieved and prevention of disease recurrence. The clinical effects of SRP indicate that SRP decreases probing pocket depth and enables attachment level gain (3). Classical mechanical treatment, SRP, enables stabilisation of most cases of periodontitis. However, in the case of tissue invasion by periodontal pathogens, such as *Porphyromonas gingivalis* (Pg) or *Actinobacillus actinomycetemcomitans* (Aa), mechanical therapy is not sufficient to eliminate bacteria from the pocket. Therefore, despite careful treatment, rapid progression of attachment loss and alveolar bone resorption occurs. In such cases antimicrobial additional therapy may be effective.

Selection of an effective agent and the means of application depend on the composition of subgingival flora and clinical signs of periodontitis. Negative test results in most cases may be a result of disease inactivity, while the presence of periodontal pathogens indicates the risk of periodontal disease progression (4).

Microbiological diagnostic procedure has therefore justifiable indication in some forms of periodontitis, such as aggressive and severe forms of chronic periodontitis. Many studies use darkfield microscopy technique and phase contrast microscopy in monitoring therapy success after SRP. The results of these studies have shown that the number of spirochetes and motile rods was reduced after therapy, while cocci and non motile rods were increased (5,6). Other studies using cultural techniques showed lower prevalence of microorganisms, such as “black pigmented Bacteroides” or specific strains like Pg and Aa (7,8,9).

However, some other studies using similar techniques found minimal effects of SRP on the subgingival microbiota, particularly for Aa (10,11,12). More recently, the use of ELISA and DNA probe techniques have confirmed reduction in Pg by SRP (13,14).

In a study by Haffajee et al. (15) DNA-DNA hybridisation technique was used in the detection and monitoring of a reduction in periodontal pathogens in chronic periodontitis before and after SRP. Technological improvements in the field of periodontal microbiology has made it possible to evaluate a broad spectrum of bacterial strains in subgingival plaque on a large number of samples.

In recent years some investigators have successfully developed the use of polymerase chain reaction (PCR) in the field of periodontology for the detection of specific microorganisms such as Aa and Pg in bacterial plaque samples.

The aim of this study was to evaluate the microbiological and clinical effects of SRP in 28 subjects with periodontitis 3 months after therapy by the PCR method (16,17).

Material and methods

Subject and site selection

Subjects with symptoms of periodontitis attending the Department of Periodontology were included in this study. All subjects were over 20 years old, with at least 20 natural teeth and many with pocket depth greater than 4mm and attachment level greater than 3 mm.

Subjects with systemic disease and those that have taken antibiotics in the last three months were excluded from the study. A total of 28 subjects, 9 with aggressive and 19 with chronic periodontitis, were included in the study and clinical and microbiological evaluation was performed prior to and after therapy.

Clinical procedures

Clinical parameters, bacterial plaque, gingival bleeding, pocket depth and attachment level were assessed on 6 teeth. Bacterial plaque and gingival bleeding were recorded as present or absent (0/1). Pocket depth and attachment level were measured using a standard periodontal probe. After taking a sample of subgingival plaque from periodontal pockets, initial therapy was performed. SRP was

performed under local anesthesia. Three months after therapy microbiological samples were again taken.

Statistical analysis

Differences between the tested parameters (plaque, gingival bleeding, pocket depth, attachment loss and bacterial prevalence) before and after therapy scaling and root planing (SRP) were tested by Wilcoxon Signed Rank Test.

Microbiological procedures

Microbiological samples were taken by sterile paper points and transported in Eppendorf tubes containing 1.5 mL of sterile digestion buffer (50 mM Tris-Cl, mM EDTA, 0.5% Tween 20, pH 8.5) and kept frozen until tested (18). The presence of bacteria was detected by DNA extraction and specific polymerase chain reaction (PCR). The cells were removed from the paper point by vigorous vortexing and proteinase K was added in a final concentration of 100 µg/ml. The samples were incubated with moderate shaking overnight at 37°C. The suspension was aliquoted (2x750µl) and total genomic DNA was further extracted by two phenol/chloroform extractions and precipitated at -20°C overnight by adding 2V of absolute ethanol. The pellet obtained after 10 minutes centrifugation at 10000 x g at room temperature was dissolved in 15-20 µL of TE buffer, pH 8.0. The quality and quantity of extracted DNA was checked by horizontal electrophoresis in 1% agarose gel stained with ethidium-bromide. The quality of DNA was estimated according to molecular weight standard (19).

Seven different pairs of primers were used in order to detect the presence of DNA from Aa, Pg, *Bacteroides forsythus* (Bf), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn), *Streptococcus mitis* (Sm) and *Leptotrichia buccalis* (Lb). For the first four bacteria primer pairs were chosen according to published data (20,21). For the rest the primers were constructed in the Division of Molecular Medicine "Ruđer Bošković" Institute, according to DNA sequence data published in Gene Bank. Two to four microliters of each DNA solution

was subjected to PCR in a total volume of 12.5 µL containing 10 mM Tris-Cl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 µM deoxynucleotide, 10 pmol oligonucleotide and 0.75 U of Taq polymerase. In short, 35 cycles at 96°C for 30 sec, 58°C for 30 sec and 72° for 40 sec were performed in a Perkin Elmer ThermoCycler 2400. Six microliters of amplified fragment were further taken to visualize the specific products in 2% gel stained with ethidium bromide. The quality of each DNA sample was checked by amplifying the segment of human, β-globin gene. This approach diminished the occurrence of false negative results which may be caused by inhibitors present in the DNA solution.

Results

In the total sample of 28 there were 9 male and 19 female subjects. The sample distribution according to diagnosis was: 19 subjects with chronic periodontitis and 9 with aggressive periodontitis. Mean plaque value was 0.94, gingival bleeding value 0.98, mean pocket depth 3.9 mm and attachment loss 4.1 mm.

Figure 1 shows the effect of scaling and root planing on clinical parameters of periodontitis. After the treatment mean plaque value decreased from 0.94 to 0.72, and gingival bleeding from 0.98 to 0.75 after the treatment. Figure 2 shows the results of mean pocket depth values and attachment loss. Mean pocket depth value was significantly decreased from 3.9 to 3.0 mm, while the decrease of attachment loss was moderate, from 4.1 to 3.8mm.

The prevalence of subgingival pathogens for each patient was as follows: only one species was found in 28.6%, two in 46.4% and three in 14.3% of subjects. In one subject four species were found and in two five species. Accordingly, the most frequent finding was the two species of subgingival pathogens.

The prevalence of subgingival species tested prior to and three months after SRP therapy are shown on figure 3. Results of the analysis of the incidence of bacteria showed that in all subjects Aa was found initially in 32.1% and after the therapy in 25% of subjects, indicating that Aa was eliminated in 7.1% of subjects. Prior to therapy Pg was

present in 32.1% subjects, and after therapy in 28.6%, showing elimination in only 7.1% of subjects. The prevalence of Pi was 17.9% before therapy, and 10.7% after therapy showing elimination in 7.2% of subjects. Bf was the most prevalent microorganism, present in 85.7% of subjects, which after treatment was eliminated in only 2 (7.1%) subjects. The prevalence of *Fusobacterium nucleatum* was 32% before therapy, and 17% after therapy. *Streptococcus mitis* and *Leptotrichia buccalis* were found in 3.6% of subjects before treatment, and were eliminated in only one patient. The prevalence of all tested species decreased in 12 subjects and this finding was statistically significant ($p = .001$). In 16 subjects no change in the number of bacteria occurred, and in no subjects did the number of bacteria increase.

Discussion

The aim of this study was to assess clinical and microbiological changes three months after initial therapy, scaling and root planing (SRP) in 28 subjects with chronic and aggressive periodontitis. The subjects were observed as a total sample due to the fact that no differences in parameters were found between the diagnoses. The results showed clinical improvement in the form of decreased gingival bleeding and bacterial plaque. Mean pocket depth values and attachment loss were markedly decreased which indicates success of the mechanical therapy of scaling and root planing. The results of this study can be compared with the results of other studies describing clinical improvement during achievement of periodontal stability (3,10,22,23). The clinical changes found in this study can be connected with changes in subgingival pathogens.

The most prevalent pathogens before therapy were: Bf, Pg, Aa, Fn and Pi. Two susceptible pathogens, *Leptotrichia buccalis* and *Streptococcus mitis*, were found in only two subjects. After the initial therapy the percentage of the above pathogens was decreased. In 12 subjects the prevalence of the examined pathogens was significantly lower, in 16 it remained the same, while no increase occurred in any subject.

The most prevalent periodontal pathogen was Bf, and all examined subjects had at least one of the examined pathogens. The decrease in the total number of pathogens after therapy was statistically significant ($p=0.01$). Similar investigations conducted over a longer period on the effect of scaling and root planing showed a decrease in prevalence and level of subgingival pathogens three months after therapy. This condition was retained, with only slight changes, during the following period of maintaining the success achieved by the initial therapy.

Conclusion

As SRP is the most prevalent form of initial periodontal therapy in the initial phase and the maintenance phase, the procedure cannot achieve the optimal effect in all cases. This is particularly the case with regard to deep periodontal pockets and complicated intraosseal defects when periodontal surgical procedures are necessary as well as antimicrobial agents.

Results of this study, seen in the clinical and microbiological improvement, indicate the effectiveness of scaling and root planing in the initial phase of therapy of periodontitis.