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The Effects of Mechanical Treatment on Microbiological Parameters in Periodontal Disease

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ABSTRACT

Bacterial plaque has the primary etiologic role in triggering the pathological changes of periodontal disease. A major goal of periodontal therapy is supra- and subgingival bacterial flora reduction through scaling and root planning, through local and general antimicrobial treatment. The aim of our study was to evaluate the effectiveness of the mechanical treatment of scaling and root planning in reducing or suppressing bacterial species from the periodontal pockets. In order to conduct this study we collected and analyzed subgingival plaque samples taken from the 50 periodontal pockets with a depth of about 5mm, from 50 subjects with diagnosis of generalized chronic periodontitis, before and after scaling and root planning. The usage of API 20A test allows a quick and easy identification of anaerobic bacteria based on biochemical properties. Additional complementary tests were used, such as examining the culture and the morpho-tinctorial features to confirm and complete the identification. The microbial flora that we were able to isolate from the periodontal pockets before scaling and root planning was very rich. After scaling and root

planning the subjects showed clinical improvement in the periodontal status, and the microbiological analysis of the periodontal pockets mostly showed a quantitative and qualitative reduction of bacterial species. A local or general antimicrobial treatment is recommended to assure improved effectiveness because mechanical treatment alone cannot completely suppress bacterial flora.

Keywords: scaling and root planning, chronic periodontitis, anaerobic bacteria

Introduction

Bacterial plaque has the primary etiologic role in triggering the pathological changes of periodontal disease. Among the subgingival plaque microorganisms some species are considered to be periodontal pathogens: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga ochracea*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Treponema denticola*, and *Campylobacter recta*.

A major goal of periodontal therapy is supra- and subgingival bacterial flora reduction through scaling and root planning by local and general

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antimicrobial treatment.

The aim of our study was to evaluate the effectiveness of the mechanical treatment of scaling and root planning in reducing or suppressing bacterial species from the periodontal pockets.

Materials and methods

In order to conduct this study we collected and analyzed subgingival plaque samples taken from the 50 periodontal pockets with a depth of about 5mm before and after scaling and root planning.

The 50 subjects included in this study were selected from patients who presented at the Department of Periodontology of the Faculty of Dentistry, the University of Medicine and Pharmacy of Tîrgu Mureş. Only patients who fulfilled the following basic criteria were included:

- diagnosis of generalized chronic periodontitis;
- the presence of periodontal pockets with a depth greater than or equal to 5 mm;
- age between 20 and 45 years, regardless of sex;
- cooperative patients.

The exclusion criteria were the following:

- the presence of systemic diseases;
- poor oral hygiene after oral hygiene instruction;
- smoking;
- pregnancy or breastfeeding;
- periodontal treatment within the last 6 months;
- antibiotic therapy in the last year.

All patients were informed about the protocol, the benefits and risks of participating in the study, and informed consent of the patients was obtained. The study was approved by the ethics committee of scientific research of the University of Medicine and Pharmacy of Tîrgu Mureş.

Each patient was subjected to prophylaxis sessions for the removal of supragingival dental

deposits and for training on individual measures to prevent plaque build-up.

The following indices for each patient were recorded in an observations sheet: gingival index, papillary bleeding index and probing depth in all odonto-periodontal units.

In each patient, we randomly selected one periodontal pocket with a depth of about 5mm from which we collected the subgingival plaque.

Mechanical treatment was performed subsequently (scaling and root planning) on the arcade which included the explored odonto-periodontal unit. Postoperative wound was protected for 7 days with a periodontal dressing.

After removal of the periodontal dressing, the new clinical indicators were recorded, and a sample was collected from the same pockets that had been explored initially.

The pathological samples collected from each periodontal pocket before and after subgingival curettage were subjected to microbiological analysis, according to the following protocol:

- after harvesting the pathological sample from the apical portion of the periodontal pocket with a Gracey curette, the sample was placed on two sterile swabs. The first swab was used for two smears - one for Gram stain and one for Giemsa staining. The second swab was used for cultivation in Schaedler broth, which was covered with a layer of melted sterile paraffin with a thickness of 5-10 mm.

- these latter samples with Schaedler broth were immediately sent to the Microbiology Laboratory, the Department of Microbiology, the University of Medicine and Pharmacy of Tîrgu Mureş, where they were incubated in the thermostat at 37°C under anaerobic conditions for 48 h;

- the Schaedler broth cultures were used for:
 - a smear for Gram stain;
 - Schaedler agar cultivation (with vitamin K and 5% sheep blood) in duplicate;
 - anaerostat incubation under anaerobic conditions (48 h);
 - anaerostat incubation in CO₂ atmosphere (48 h).

Smears were performed from the microbial colonies grown in each of the two Schaedler agar environments and the bacterial species were

identified using the API 20A (bioMerieux) manual identification galleries. Strips which consists of 20 microtubes containing dehydrated substrates.

Usage of API 20A test allows a quick and easy identification of anaerobic bacteria based on biochemical properties. Additional complementary tests were used, such as examining the culture and the morpho-tinctorial features to confirm and complete the identification.

After obtaining the pure culture or isolated colonies of bacteria to be identified, by means of a sterile swab we collected a few colonies in which we performed a suspension in API 20A medium, the turbidity of which was equal to that of tube 3 on the McFarland scale.

From this suspension the needed quantities were introduced in microtubes with a sterile pipette. The IND tube was covered with a layer of paraffin oil. The strip was then incubated under anaerobic conditions in an anaerostat for 24-48 hours at 37°C. Following crop growth, production of metabolites was evidenced by color changes of pH indicators or by adding additional reagents after incubation.

Results

Biochemical reactions that can be performed using API 20A strips galleries are: the indole synthesis, urease production, decomposition of sugars (glucose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose), gelatin hydrolysis, esculin hydrolysis, decomposition of glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, trehalose, the production of catalase.

The results were recorded on charts and the characteristic values for each species were calculated using the identification scheme.

These results were completed by microscopic examination of the Gram smear performed from the culture, where we noted the presence or absence of spores, tinctorial affinity (Gram-positive or Gram-negative) and morphology (cocci or bacilli).

On the Gram smears with subgingival plaque taken from the periodontal pockets prior to the subgingival curettage we observed increased percentage of Gram-negative bacilli of various lengths, some of which were grouped in chains, others with unequal diameter and uneven staining. Gram-positive and Gram-negative isolated cocci, in pairs or grouped in chains, were present in small numbers. Giemsa staining allowed us to highlight spiral shapes and the presence of large numbers of erythrocytes and epithelial cells on these smears (Table I).

Following scaling and root planning, the Gram and Giemsa smears with biological product harvested from the same pockets examined initially were characterized by increased spread of Gram-positive and Gram-negative cocci at the expense of polymorphic Gram-negative bacilli. (Table I)

The Schaedler agar smears with subgingival plaque taken from the periodontal pockets prior to the subgingival curettage allowed us to note the presence of large numbers of bacterial colonies, most of them having a brown/ black pigmentation, with or without hemolysis.

Table I - Comparison of subgingival plaque smears (Gram and Giemsa stains)

Smear	Prior the treatment	After the treatment
Streptococcus spp.	+/++	++/+++
Staphylococcus spp.	+	+
Neisseria spp.	+	+
Veillonella spp.	+	+
Lactobacillus spp.	+	++
Polymorphic Gram-negative bacilli	++/+++	+
Fusobacterium spp.	++	+
Campylobacter spp.	++/+++	+/++
Treponema spp.	+/++	+
Erythrocytes, leucocytes, epithelial cells	++/+++	+

Key: +: low number, ++: great number, +++: very great number

Microbial colonies grown in each of the two Schaedler agar environments were used to prepare

smears and to identify periodontal bacterial species known to be periodonto –pathogenic using the API 20A (bioMerieux) manual identification galleries.

After scaling and root planning most periodontal pockets showed a reduction in the quantity and quality of the previously detected bacterial species (Table II).

Discussions

The API 20A (bioMerieux) manual identification galleries allowed us to identify microbial species present in the periodontal pockets.

The microbial flora that we were able to isolate from the periodontal pockets before scaling and root planning was very rich. Among the plaque microorganisms taken from patients with chronic marginal periodontitis, we isolated bacterial species known to be responsible for the pathological changes that accompany these conditions: *Porphyromonas gingivalis* (Figure 1), *Prevotella melaninogenica/oralis*, *Veillonella parvula*, *Fusobacterium mortiferum*, *Streptococcus intermedius*, *Peptostreptococcus asaccharolyticus*, and *Bacteroides distasonis*. *Aggregatibacter actinomycetemcomitans* was not evidenced in any of the patients included in our study because the API 20A (bioMerieux) manual identification galleries do not identify this organism.

After scaling and root planning the subjects showed clinical improvement in the periodontal status, and the microbiological analysis of the periodontal pockets mostly showed a reduction of quantitative and qualitative bacterial species.

Among anaerobic bacteria detected prior to the treatment *Prevotella melaninogenica/ oralis* was the organism that was most evident in a significantly reduced number of patient safter scaling and root planning.

There were instances where certain bacterial species, most frequently *Porphyromonas gingivalis*, were present in the periodontal pockets after mechanical treatment, although the marginal periodontium seemed clinically healthy, which

was explained by the increased virulence of this microorganism.

Table II. Isolated anaerobic bacteria in patients before and after the treatment

Anaerobic bacteria	Prior to the treatment	After the treatment
<i>Porphyromonas gingivalis</i>	19 cases	14 cases
<i>Prevotellamelaninogenica/oralis</i>	16 cases	2 cases
<i>Fusobacterium mortiferum</i>	3 cases	-
<i>Streptococcus intermedius</i>	5 cases	3 cases
<i>Peptostreptococcus asaccharolyticus</i>	4 cases	3 cases
<i>Veillonella parvula</i>	3 cases	-
<i>Bacteroides distasonis</i>	2 cases	-

The therapeutic value of the mechanical treatment (scaling and root planning) was assessed by numerous studies by quantifying the presence and number of these microorganisms before and after the treatment [1-4]

Some authors [5-7] suggest that periodontal therapy outcomes are better when *Porphyromonas gingivalis* and *A.actinomycetemcomitans*, the major periodontal pathogens, are not detected or are present in low numbers after the treatment.

Figure 1 - The API 20A gallery used for the identification of Porphyromonas gingivalis

To strengthen the clinical and microbiological results obtained after mechanical treatment particular attention must be given to the maintenance period. [8-12]

Because obtaining a periodontal pocket without any pathogenic periodontal microorganisms

by scaling and root planning alone is hard to attain, it is recommended that a local [13-15] or general adjuvant antimicrobial treatment [16-19] be used.

Conclusion

Reduction of the subgingival bacterial flora by scaling and root planning by local and general antimicrobial treatment is a major goal of periodontal therapy.

After scaling and root planning our subjects showed improvement in the periodontal status and the microbiological analysis of most periodontal pockets showed a reduction of quantitative and qualitative bacterial species detected prior to the treatment.

A local or general antimicrobial treatment is recommended to assure improved effectiveness because mechanical treatment alone cannot completely suppress bacterial flora.

References

1. Takamatsu, N., Yano, K., He, T., Umeda, M. & Ishikawa, I. (1999). Effect of initial periodontal therapy on the frequency of detecting *Bacteroides forsythus*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. *J Periodontol.* 70(6), 574-580
2. Ali, R.W., Johannessen, A.C., Dahlen, G., Socransky, S.S. & Skaug, N. (1997). Comparison of the subgingival microbiota of periodontally healthy and diseased adults in northern Cameroon. *J Clin Periodontol.* 24(11), 830-835
3. Mombelli, A., Schmid, B., Rutar, A. & Lang, N.P. (2000). Persistence patterns of *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, and *Actinobacillus actinomycetemcomitans* after mechanical therapy of periodontal disease. *J Periodontol.* 71(1), 14-21
4. Doungudomdacha, A., Rawlinson, A., Walsh, T.F. & Douglas, C.W.I. (2001). Effect of non-surgical periodontal treatment on clinical parameters and the numbers of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* at adult periodontitis sites. *J Clin Periodontol.* 28, 437-445
5. Haffajee, A.D., Cugini, M.A., Dibart, S., Smith, C., Kent, R.L. Jr. & Socransky, S.S. (1997). The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *J Clin Periodontol.* 24(5), 324-334
6. Slots, J. & Ting, M. (2000). *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. *Periodontol.* 20, 82-121
7. Darby, I.B., Mooney, J. & Kinane, D.F. (2001). Changes in subgingival microflora and humoral immune response following periodontal therapy. *J Clin Periodontol.* 28(8), 796-805
8. Shiloah, J., Patters, M.R., Dean, J.W., Bland, P. & Toledo, G. (1998). Prevalence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Bacteroides forsythus* in humans 1 year after 4 randomized treatment modalities. *J Periodontol.* 69, 1364-1372
9. Petersilka, G.J., Ehmke, B. & Flemmig, T.F. (2002). Antimicrobial effects of mechanical debridement. *Periodontol.* 28, 56-71
10. Jorgensen, M.G. & Slots, J. (2002). The ins and outs of periodontal antimicrobial therapy. *J Calif Dent Assoc.* 30(4), 297-305
11. Cugini, M.A., Haffajee, A.D., Smith, C., Kent, R.L. & Socransky, S.S. (2000). The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases: 12 month results. *J Clin Periodontol.* 27, 30-36
12. Ximénez - Fyvie, L.A., Haffajee, A.D., Som, S., Thompson, M., Torresyap, G. & Socransky, S.S. (2000). The effect of repeated professional supragingival plaque on the composition of the supra- and subgingival microbiota. *J Clin Periodontol.* 27(9), 637-647

13. Paolantonio, M., D'Angelo, M., Grassi, R.F., Perinetti, G., Piccolomini, R., Pizzo, G., Annunziata, M., D'Archivio, D., D'Ercole, S., Nardi, G. & Guida, L. (2008). Clinical and microbiologic effects of subgingival controlled-release delivery of chlorhexidine chip in the treatment of periodontitis: a multicenter study. *J Periodontol.* 79(2), 271-82
14. Sakellari, D., Ioannidis, I., Antoniadou, M., Slini, T. & Konstantinidis, A. (2010). Clinical and microbiological effects of adjunctive, locally delivered chlorhexidine on patients with chronic periodontitis. *J Int Acad Periodontol.* 12(1), 20-26
15. Gonzales, J.R., Harnack, L., Schmitt-Corsitto, G., Boedeker, R.H., Chakraborty, T. & Domann E. et al. (2011). A novel approach to the use of subgingival controlled- release chlorhexidine delivery in chronic periodontitis: a randomized clinical trial. *J Periodontol.* 82, 1131-1139
16. Matesanz, P., Herrera, D., Echeverría, A., O'Connor, A., González, I. & Sanz, M. (2012). A randomized clinical trial on the clinical and microbiological efficacy of a xanthan gel with chlorhexidine for subgingival use. *Clin Oral Investig.* 16
17. Walker, C.B., Karpinia, K. & Baehni, P. (2004). Chemotherapeutics: Antibiotics and other antimicrobials. *Periodontol.* 36, 146–165
18. Guerrero, A., Griffiths, G.S., Nibali, L., Suvan, J., Moles, D.R. & Laurell L. et al. (2005). Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: A randomized placebo controlled clinical trial. *J Clin Periodontol.* 32, 1096–1097
19. Kapoor, A., Malhotra, R., Grover, V. & Grover, D. (2012). Systemic antibiotic therapy in periodontics. *Dent Res J.* 9(5), 505–515