

BACTERIOLOGICAL FINDINGS DURING THE TREATMENT OF CHRONIC AND AGGRESSIVE PERIODONTITIS USING INITIAL THERAPY

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ABSTRACT

Objective. The aim of this study was to determine the bacteriological substrate of periodontal pathogens in different time intervals in patients with aggressive and chronic periodontitis undergoing the initial therapy.

Materials and methods. The research was conducted on 40 patients (25 male, 15 female) who had received no prior treatment, with the average age of 39. The patients were divided in two groups: Group I patients with aggressive periodontitis (n=18), with the average age of 34.5 years, Group II patients with chronic periodontitis consisted (n=22), with the average age of 43. The microbiological analysis of subgingival biofilm was performed on baseline day, 7 and 30 days after the treatment.

Results. Our findings show the following distribution of bacteria in patients with aggressive periodontitis: *A. actinomycetemcomitans* 72.2%, *P. gingivalis* 50%, *F. nucleatum* 44.4%, *T. forsythia* 44.4% and *P. intermedia* 16.7%. In patients with chronic periodontitis the following anaerobic bacteria were identified: *P. intermedia* 63.6%, *F. nucleatum* 54.5%, *A. actinomycetemcomitans* 36.4%, *P. gingivalis* 36.4% and *T. forsythia* 22.7%.

Conclusion. The findings of this research show that the levels of specific periodontal subgingival pathogens were lower after the initial therapy and indicate the necessity and appropriateness of the initial periodontal therapy. The microbiological identification of periodontal bacteria proved to be of immense importance since it can help to select the right antibiotic or the combination of antibiotics.

Key words: initial therapy, bacteriological substrate, aggressive and chronic periodontitis.

Introduction

Periodontal diseases and caries are the most widely spread dental diseases of our time. Periodontitis is a chronic infection caused by gram-negative bacteria affecting the tissues that support the teeth [1]. Microorganisms, the main cause of periodontal disease, colonize the surface of the teeth above and below the gingival margin. These differences are the result of different amounts of blood products and nutrients in different areas. But, the difference in composition can also be attributed to pocket depth, redox potential and pO₂.

Periodontal diseases are caused by a group of periodontal pathogens acting alone or together. The bacterial flora of the oral cavity is a complex one. The oral cavity is assumed to host between 300 and 1,200 different bacterial species, 50% of which is impossible to cultivate [2, 3]. At least 400 different bacterial species live in subgingival areas, and 20 of them are believed to be the most powerful periodontal pathogens [4, 5]. The gram-negative anaerobes such as *Aggregatibacter actinomycetemcomitans* (*A.a*), *Tannerella forsythia* (*T.f*), *Fusobacterium nucleatum* (*F.n*), *Porphyromonas gingivalis* (*P.g*) and *Prevotella intermedia* (*P.i*) are considered to be the most probable cause of periodontitis [6].

Periodontal disease is also called a mixed bacterial infection, so as to emphasize that more than one species of bacteria play a role in the advancement of the disease. Chronic periodontitis (CP) progresses slowly and occurs in people of middle and older age as a localized and generalized form of the disease. The amount of the plaque correlates with the degree of inflammation as well as the degree of periodontal destruction. Subgingival plaque and gingival recession are typical clinical findings for chronic periodontitis [7, 8]. Aggressive periodontitis (AgP) usually occurs in younger people. It is often related to a genetic predisposition and impaired inflammatory-immune response. A response of the host in the form of an immune reaction is not powerful enough thus contributing to a faster progression of the disease [9]. Clinically, there is not much plaque or subgingival plaque to be found, attachment loss and usually vertical bone defects occur, and the progress of the disease cannot be predicted [10,11].

Microbiological tests are particularly important for assessing the need for an additional antibiotic

therapy, selecting an antimicrobials medicine and estimating the effects of the treatment. Microbiological information can, therefore, be useful in different stages of the therapy such as the initial diagnosis, reevaluation and recall phase.

The purpose of this study was to determine the bacteriological substrate of periodontal pathogens in different time intervals in patients with aggressive and chronic periodontitis using the initial therapy.

Materials and methods

The research was conducted on 40 periodontal patients with no prior treatment, 25 males and 15 females, admitted to the Department/Clinic for Periodontology and Oral Medicine of the Faculty of Dentistry at the University of Sarajevo. The first group comprised of 18 patients diagnosed with AgP, with the average age of 34.5. The other group comprised of 22 patients diagnosed with CP, with the average age of 43. Patients with systemic disorders, as well as those who had taken antibiotics and antiseptics in the month prior to the research, were excluded from the research.

The clinical periodontal exam included the measurement of periodontal pockets (PD), clinical attachment loss (CAL), sulcus bleeding index (SBI), plaque index (PI), looseness of teeth and RTG analysis. Prior to the beginning of the treatment, the patients were thoroughly instructed in proper oral hygiene. The initial periodontal treatment was exclusively performed with the EMS Mini Piezon ultrasonic scaler with frequency range of 25 – 32 kHz. During the course of the study we did the microbiological identification of 5 periodontal pathogenic bacteria prior to the beginning of the therapy, 7 days and 30 days after the beginning of the initial periodontal therapy. Swab samples were obtained from the deepest periodontal pockets. The samples were collected with endodontic paper points (size 55) which were inserted to the bottom of the periodontal pockets and kept in place for 30 seconds. The samples were placed in a transport medium with Amies agar gel, providing the conditions necessary for the life of anaerobic microorganisms. The anaerobic plate count was used for the identification of the anaerobic microorganisms. The morphology of the grown colonies was also analyzed.

Agressive periodontitis (n=18 patients)						
	Before treatment		7 days after treatment		30 days after treatment	
<i>Aggregatibacter actinomycetemcomitans</i>	13	72,22%	9	50,00%	6	33,33%
<i>Porphyromonas gingivalis</i>	9	50,00%	4	22,22%	4	22,22%
<i>Prevotella intermedia</i>	3	16,67%	2	11,11%	4	22,22%
<i>Tannerella forsythia</i>	8	44,44%	3	16,67%	2	11,11%
<i>Fusobacterium nucleatum</i>	8	44,44%	3	16,67%	5	27,78%

Table 1. Distribution of the bacteria of AgP in the three different time intervals

Statistical analysis

All statistical analyses were done in Windows SPSS v.15 at the level of statistical significance of $p < 0.05$. The presence of bacteria of aggressive and chronic periodontitis from the samples obtained in three different time intervals is summarized by descriptive statistics. The importance in value change in the bacteria before and after the treatment was tested by the T-test. Tests were also carried out to check the normality of the distribution of results, using the Shapiro-Wilk test for small samples (up to 50), then followed by the ANOVA test of variance, Levene's test of homogeneity of variance and, finally, Dunnett's T3 multiple comparisons procedure.

Results

Aggressive periodontitis

Presented in **Table 1** are our results showing the distribution of the bacteria of AgP in three different time points.

Out of 18 patients, 13 (72.22%) had tested positive for *A.a* before the initial periodontal treatment, while 6 (33.33%) still tested positive for *A.a* even 30 days after the beginning of the treatment. 9 (50%) patients had tested positive for *P.g*, however, after 30 days the number decreased to 4 (22.22%). *P.i* was isolated in 3 (16.67%) patients with AgP, while 4 (22.22%) patients still tested positive for *P.i* 30 days after the beginning of the therapy. In our research, 8 (44.4%) patients with AgP tested positive for *T.f* and *F.n* 30 days after the beginning of the treatment 2 (11.11%) patients with AgP tested positive for *T.f* and 5 (27.78%) patients for *F.n* (**Figure 1, 2, 3**).

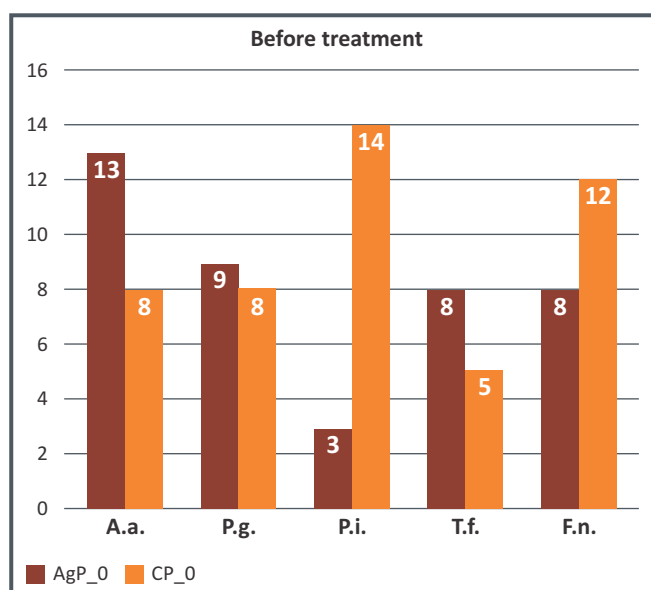


Figure 1. Patients with the bacteria of AgP and CP before the treatment

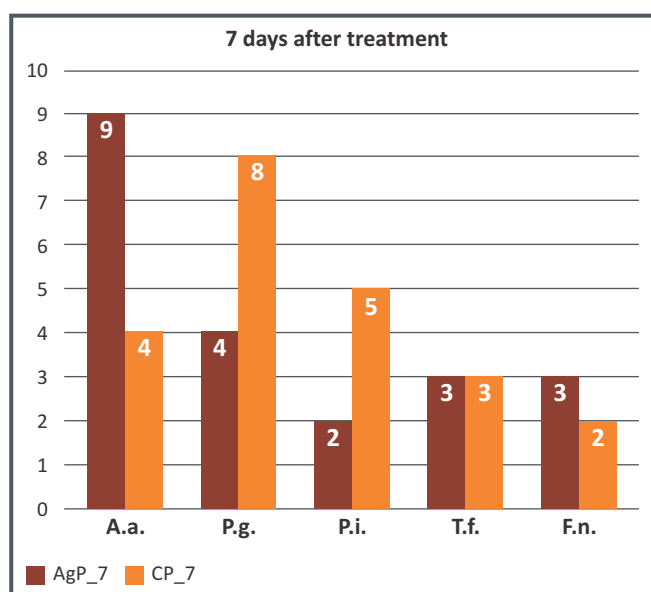


Figure 2. Patients with the bacteria of AgP and CP 7 days after the beginning of the treatment

The one-way Anova test shows no statistically significant differences in distribution of AgP bacteria in three time intervals ($p=0.062$) (Table 2).

Chronic periodontitis

Our findings on distribution of the bacteria of CP in the three time intervals are presented in Table 3.

8 (36.36%) patients out of 22 with CP had tested positive for *P.g* before the treatment, and only 4 (18.1%) tested positive 30 days after the beginning of the treatment. The same number had tested positive for *P.g* before the treatment, and only 2 (9.10%) patients 30 days after the beginning of the treatment.

In our study 14 (63.63%) patients with CP had tested positive for *P.i* before the treatment, and 30 days after the beginning of the treatment only 5 (22.73%) tested positive. 5 (22.73%) patients had tested positive for *T.f* and 30 days after the beginning of the treatment the therapy proved effective and all the patients tested negative for *T.f*. 12 (54.55%) patients had tested positive for *F.n* and 30 days after the beginning of the therapy only 4 (18.18%) patients with CP tested positive for *F.n* (Figure 1, 2, 3).

The one-way Anova test showed that there was a statistically significant difference in distribution of chronic periodontitis bacteria in the three time intervals ($p=0.007$) (Table 4).

Dunnett's T3 tests showed the difference between the first (initial) and third time intervals (30 days after the beginning of the treatment) ($p=0.0033$, $p<0.05$), in a sense that the number of patients who tested positive for the bacteria decreased 30 days after the beginning of the treatment. The numerical relationship between the presence of AgP and CP bacteria in the time intervals of scaling and root

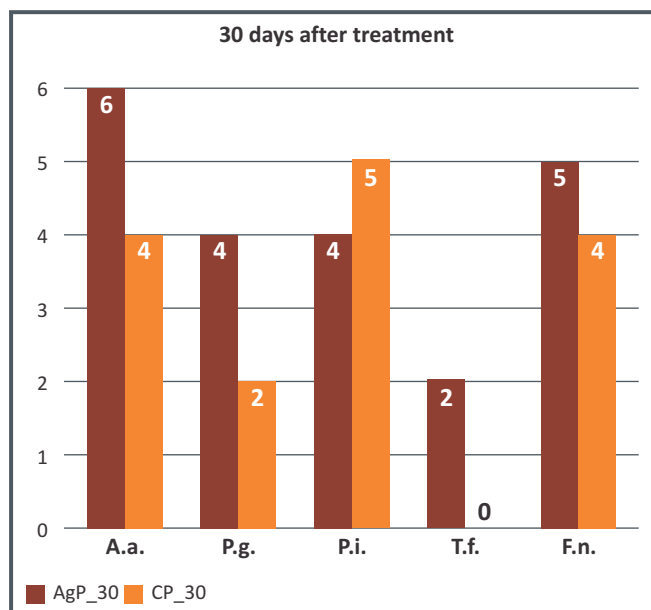


Figure 3. Patients with the bacteria of AgP and CP 30 days after the beginning of the treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53,333	2	26,667	3,540	0,062
Within Groups	90,400	12	7,533		
Total	143,733	14			

Table 2. The examination of differences in the distribution of the bacteria of AgP in the three time intervals

Chronic periodontitis (n=22 patients)						
	Before treatment		7 days after treatment		30 days after treatment	
Aggregatibacter actinomycetemcomitans	8	36,36%	4	18,18%	4	18,18%
Porphyromonas gingivalis	8	36,36%	8	36,36%	2	9,10%
Prevotella intermedia	14	63,63%	5	22,73%	5	22,73%
Tannerella forsythia	5	22,73%	3	13,64%	0	0,00%
Fusobacterium nucleatum	12	54,55%	2	9,10%	4	18,18%

Table 3. The distribution of the bacteria of CP in the three time intervals

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	113,200	2	56,600	7,683	0,007
Within Groups	88,400	12	7,367		
Total	201,600	14			

Table 4. The examination of differences in the distribution of the bacteria of CP in the three time intervals

planning with hand and ultrasound instruments to treatment, 7 days and 30 days after the beginning of the treatment is shown in the chart (**Table 5**).

T-test for independent samples was used to test differences in patients with AgP and CP in three time intervals. The results showed no significant statistical differences.

Discussion

The aim of this paper was to determine the bacteriological substrate of periodontal pathogens in different time intervals in patients with aggressive and chronic periodontitis using the scaling and root planning with hand and ultrasound instruments. During the course of our study, 5 periodontal pathogenic bacteria were identified before the treatment, 7 days and 30 days after the beginning of the initial periodontal treatment. These were *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*. Majority of subgingival bac-

teria are sensitive to antimicrobial effects of mechanical debridement [12]. Mechanical debridement combined with a proper oral hygiene can in most patients stop and prevent the further loss of periodontal attachment as well as the further progression of periodontal disease. Scientific research shows that the same results can be achieved with ultrasonic instruments as with scrapers and curettes, in addition to saving time and being well-accepted among the patients.

Thanks to new-generation inserts with thinner tips the indication field of ultrasonic instruments has expanded. Today, the concept of initial therapy undergoes a change and the new concept is founded on the belief that ultrasonic instruments could be perceived as an ultimate approach to one segment of periodontal therapy. The most important argument in favor of microbiological identification of periodontal pathogens prior to antibiotic therapy is the fact that a specific antibiotic, or a group of antibiotics, may be used to identify the bacteria.

During our microbiological analysis we have determined the presence of *A. actinomycetemcomitans* depending on the confirmed diagnosis of AgP or CP. The total number of patients with AgP was 18, while the total number of those with CP was 22. Aa was more present in patients diagnosed with AgP than with CP, that was in accordance with previously published data [14]. We can conclude that *A. actinomycetemcomitans* is more often present in patients diagnosed with AgP (72.22%) than in patients diagnosed with CP (36.36%). In our research we found that *P. gingivalis* is more common in patients with AgP (50%) than in patients with CP (36.36%) which is consistent with the findings of Kadkhod et al. [15].

(I) var_mjer_CP	(J) var_mjer_PH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Upper Bound	Lower Bound
CP_before_th	CP_th_7	5,00000	1,90263	0,092	-0,8519	10,8519
	CP_th_30	6,40000(*)	1,83303	0,033	0,6273	12,1727
CP_th_7	CP_pre_th	-5,00000	1,90263	0,092	-10,8519	0,8519
	CP_th_30	1,40000	1,36382	0,677	-2,6520	5,4520
CP_th_30	CP_pre_th	-6,40000(*)	1,83303	0,033	-12,1727	-0,6273
	CP_th_7	-1,40000	1,36382	0,677	-5,4520	2,6520

Table 5. Dennett T3 test of the examination of differences in the bacteria of CP in the three time intervals

Likewise, Decaillet et al. have come to a conclusion about the relationship between *A. actinomycetemcomitans* and *P. gingivalis*. Out of 70 samples of subgingival plaque, 67 tested positive for *A. actinomycetemcomitans* and *P. gingivalis*. In other words, had *A. actinomycetemcomitans* been isolated in patients, we could have expected to isolate *P. gingivalis* as well [16].

Based on their microbiological research in chronic periodontitis, Cionca et al. have concluded that if *P.g* was to be isolated from a plaque sample, *T.f* and *T.d* could also be expected to be isolated from the same sample [17]. In our study, a higher percentage of *P. intermedia* was found in 14 (63.33%) patients with CP, than in 3 (16.67%) patients with AgP. In that research, *P.i* was isolated in over 50% of the patients with CP. In our research, before the therapy, the higher level of *T.f* was found in 8 (44.44%) patients with AgP than in 5 (22.73%) patients with CP. 30 days after the beginning of the therapy all 22 patients tested negative for *T.f* and the therapy proved successful. *T.f* appeared to be related to CP, which is inconsistent with the results of our research [18].

In their research, Settem et al. investigated the synergistic effect of *T.f* and *Fn* on the resorption of alveolar bone. They proved that mixed periodontal infections caused by these two bacteria lead to a faster absorption of alveolar bone than infections caused by either *T.f* or *Fn* [19]. According to the latest research, the role of *Fn* in oral hygiene is a very controversial one. In his research, Ji examined an influence of *T.n* and its cytopathogenic effects on gingival epithelial cells. *Fn* proved to be of great invasive ability, almost as invasive as *P.g*, however, with no cytopathic effect on epithelial cells [20].

Fn is considered to be an opportunistic pathogen and a key connection between the early and late colonizing bacteria in periodontal pockets, which is in line with our findings where a greater presence of *Fn* was found in 12 (54.55%) patients with CP than in 8 (44.44%) patients with AgP. Polak et al. investigated the possibility of the co-aggregation between *P.g* and *Fn* affecting the virulence of mixed periodontal infections. They found that using lactose as an inhibitor of *Fn* reduced pathogenicity of *P.g*, thus proving the correlation between *Fn* and *P.g* [21].

In the course of our study in patients with AgP, percentage of isolated bacteria were as follows: *A.a* 72.22%, *P.g* 50.00%, *Fn* 44.44%, *T.f* 44.44% and *P.i*

16.67%. With the patients with CP the following bacteria were found: *P.i* 63.63%, *Fn* 54.55%, *A.a* 36.36%, *P.g* 36.36% and *T.f* 22.73%. 30 days after the beginning of the initial therapy, the percentages of the examined periodontal pathogens in both types of the disease were lowered.

This study indicates that not all bacteria found in the subgingival biofilm react in the same way to the initial periodontal treatment. Some types reduce in numbers, while others persist. Negative bacteriological findings can be the result of an inactive disease, while the presence of periodontal pathogens warns about the risk of further periodontal destruction.

Conclusion

Since the scaling and root planning with hand and ultrasound instruments is the basic and the most common non-surgical periodontal therapy, in the onset phase as well as in the phase when the achieved results are to be maintained, we can conclude from our findings that the initial therapy is not always sufficient for an optimal non surgical periodontal treatment outcome. The findings of this research show that the level of specific periodontal subgingival pathogens does decrease indicating the necessity and appropriateness of the initial therapy treatment.

Microbiological identification of periodontal bacteria neither helps to establish the diagnosis nor to determine the difference between aggressive periodontitis and chronic periodontitis, but it is, however, important in the selection of antibiotics or a combination of antibiotics. The presence of periodontal pathogens in subgingival flora poses a risk for the advancement of the disease. Consequently, there is a rationale for using microbiological diagnosis to detect pathogens, monitor the success rate of the therapy as well as the outcome of the disease.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

Authors' contributions: Conception and design: PE, HS; Acquisition, analysis and interpretation of data: PE, GM and NE; Drafting the article PE, HS, GA and BE; Revising it critically for important intellectual content: PE, HS and GM.

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