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Effects of fixed orthodontic appliances on subgingival microflora

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Abstract: *Objectives:* Fixed orthodontic appliances cause plaque accumulation around bands and brackets. Since the microbiological composition of dental plaque is closely connected to periodontal tissue health, the aim of this study was to determine the effects of fixed orthodontic appliances on subgingival microflora and periodontal status. *Methods:* This prospective study was carried out on 32 adolescents scheduled for fixed orthodontic treatment. Subgingival dental plaque samples and periodontal records (pocket probing depth and clinical attachment level) were obtained in four recording times: before bonding of fixed appliances (T0), 1 (T1), 3 (T2) and 6 (T3) months after the beginning of orthodontic therapy, in order to detect the changes in periodontopathic anaerobe microbial flora and its effects on periodontal status. *Results:* The values of pocket probing depth, total number of microorganisms and number of patients with positive findings of *Prevotella intermedia* and other periodontopathic anaerobes increased from T0 to the maximum obtained in T2 recording time. Both clinical and microbiological values decreased 6 months after the beginning of orthodontic therapy. *Conclusions:* The therapy with fixed appliances may transitionally increase the growth of periodontopathogenic bacteria and consequently result in gingival inflammatory response but without destructive effect on deep periodontal tissues.

Key words: anaerobes; brackets; oral hygiene; periodontium; plaque

Introduction

Inflammatory reaction of gingival tissue appears very often during orthodontic therapy of various malocclusions with fixed

appliances. The appearance of new retentive places around bands and brackets is considered to be the main factor for an increased accumulation of dental plaque and inflammatory response (1). Based on this fact, initially, researchers had mainly concentrated on the effect of fixed orthodontic appliances on oral hygiene and gingival status (2, 3). With time, the development of microbiology resulted in an increased number of studies dedicated to the investigation of specific microbiological changes of dental plaque among patients treated with fixed appliances (4–6). Initially, cariogenic microflora, such as *Streptococcus mutans* or *Lactobacillus* spp., and subsequent teeth decalcification were the main field of interest among investigators (7, 8). Later, the efforts of researchers were aimed at a very complex subgingival flora composed of periodontopathic anaerobes (9, 10). The aim of this study was to investigate the influence of fixed orthodontic appliances on quantitative and qualitative changes of subgingival microflora and to assess the result of these microbial shifts upon periodontal tissues.

Materials and methods

Subjects

This prospective study was carried out on 32 patients of both sexes (13 males, 19 females) aged between 12 and 18 years. The adolescents were patients of the Clinic of Orthodontics, Faculty of Stomatology, University in Belgrade. All of them fulfilled the following criteria for participation in this study (4): good general and initial periodontal health, the presence of indication for fixed orthodontic therapy regardless of malocclusion type and obligatory lack of antibiotic therapy 3 months before the beginning of the study and during the study and no usage of antiplaque and oral antiseptic solutions during the entire investigation. Written parental consent was obtained prior to the study. The Ethics Committee of the Faculty of Stomatology, University in Belgrade, Belgrade, Serbia, granted ethical approval for the study.

Fixed orthodontic technique and oral hygiene

The fixed orthodontic treatment was performed with directly bonded metal brackets (Ricketts Universal 18, Ultratrimm, Dentaurum, Ispringen, Germany), applied on incisors, canines and premolars using light cure adhesive (Enlight, Ormco Corp., Orange, CA, USA), and orthodontic bands (Dentaform Bands-Preweld, Roth 18, Dentaurum, Ispringen, Germany) cemented with glass–ionomer cement (Aqua Meron, Voco GmbH, Cuxhaven, Germany) on first molars. Three weeks before starting treatment, subjects received oral hygiene instructions (tooth brushing according to the technique of Bass (1) and were supplied with adequate approximal and fixed appliance toothbrush.

Clinical procedures

The health status of periodontal tissues was determined by assessment of the pocket probing depth, measured to the nearest millimeter on a calibrated periodontal probe (Goldman-Fox, Hu-Friedy Mfg. Co., Inc., Chicago, IL, USA), and measurement of clinical attachment level. These clinical parameters were assessed on the mesio-buccal point and in the middle of oral tooth surface on the examined teeth: upper right first molar, upper left central incisor and upper left first premolar. The measurements were obtained before bonding (T0), and 1 (T1), 3 (T2) and 6 (T3) months after the beginning of orthodontic treatment. In every recording microbiological samples were collected prior to the clinical procedures.

Microbiological procedures

The material for microbiological examination was obtained from subgingival mesio-buccal points of above mentioned upper teeth. If some of the representative tooth was missing the neighbour tooth from the same teeth group was used instead. This was the case only in three patients. Two of them had the first left premolar extracted as the necessary part of the treatment plan for their malocclusion type and in those cases the second left premolar was used instead. The third patient had upper right first molar missing because of its previous extraction and therefore the upper right second molar was used for examination. Since the neighbour teeth were used only in three investigated patients it could be anticipated that this could not have an effect on the results of the study. The microbiological samples were collected in the same four periods of time as above mentioned clinical measurements. The microbiological investigation was done in order to confirm the presence or absence of periodontopathic anaerobes: *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans* and the group of other black-pigmented anaerobes such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.

The subgingival plaque samples were collected in dry field conditions by inserting two sterile paper points (ISO 45) (11) carefully to the deepest part of gingival sulcus parallel to the tooth vertical axis for 60 s. After insertion paper points were dropped into a vial containing 2 ml of reduced transport fluid brain-heart infusion (BHI; Difco Laboratories, Detroit, MI,

USA) with 5 mg hemine l added and immediately transported to the Institute of Microbiology and Immunology, University Medical School of Belgrade for further analyses. The transport medium was prepared under anaerobic conditions during 48 h before sampling and by adding 0.5 ml paraffin oil prior to the collection of subgingival plaque. After vortexing (25 Hz) for 2–3 min 100 μl of plaque samples were plated directly and also, for more precise analyses, diluted (100 μl in 2 ml of clean BHI) and plated for primo isolation on BHI agar with 5 mg hemine l, 50 mg L-cisteine l and 10% defibrinated horse blood on the temperature between 45 and 56°C (12). The incubation lasted 72 h under anaerobic conditions with Gas Pack System (bioMerieux, Marcy L'Etoile, France) following with semiquantative method for determination of anaerobic bacteria colony number using direct counting and density comparision. For the semiquantitative method the specimen was swabbed onto four quadrants of agar plate successively diluting the specimen. Agar plate was streaked on one quadrant and than on each remaining quadrant using a sterile loop for each quadrant. This procedure creates dilutions of the original swab in each quadrant. After incubation under anaerobic conditions the plates were visually inspected and the colonies of bacteria were counted in four quadrants if their number was <300. Subculturing, Gram-stain and identification tests of biochemical reactions were used for accurate identification of bacterial species. The selective subcultures were performed immediately after incubation or delayed by preserving formed bacterial colonies in BHI fluid containing 10% glycerol on the temperature of −20°C. Kanamycin-vancomycin-laked blood agar was used for selection of *P. intermedia* and other pigmented anaerobic Gram negative bacteria. Trypticase soy-serum vancomycin bacitracin agar was the selective medium for isolation of *A. actinomycetemcomitans* (13). *Prevotella intermedia* were identified by positive indole production, haemolysis on sheep blood and negative reaction for nitrate reduction.

Statistical analysis

Statistical analyses included descriptive statistical parameters and Student's *t*-test combined with Wilcoxon signed ranks *Z*-test and *Q*-Cochran test.

Results

Total bacterial count

Total bacterial count showed similar changes in all three groups of examined teeth during periods of recording. The total number of microorganisms isolated in subgingival dental plaque samples increases from the first two recordings to T2 and T3 examination periods. It could be seen that the maximum of total bacterial count was recorded in the region of incisors three months after bonding of fixed appliances (Fig. 1).

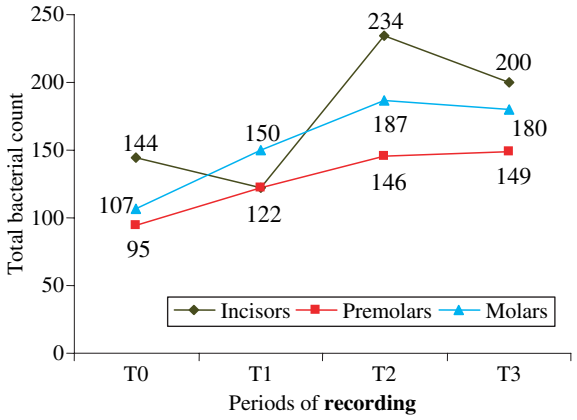

Fig. 1. The changes of total bacterial count (colony forming unit/ml) through periods of recording.

The results showed, comparing the first two recordings with values obtained 3 months after the bonding (Table 1), that the differences between the total bacterial count were statistically significant on incisors as well as on premolars and molars, but this significance was especially high for incisors region.

Periodontopathogenic anaerobes

Additionally, the number of patients with positive findings of *P. intermedia* colonies was increasing from the first recording to the maximum values showed in T2, followed by decrease after 6 months. These results were obtained in all three groups of examined teeth but the most statistically significant changes during the entire study were shown among incisors (Fig. 2). It could be seen that the frequency of positive findings of *P. intermedia* changes the most statistically significant in dental plaque collected from incisors, through periods of recording, comparing to premolars and molars (Table 2).

Actinobacillus actinomycetemcomitans, the most potent periodontopathogenic bacteria associated with aggressive periodontitis, was isolated only in one patient. The positive findings of *A. actinomycetemcomitans* were noted on incisors at T1 and T2 recordings.

Finally, the occurrence of all other black-pigmented anaerobic bacteria showed the similar changes during recordings on examined incisors, premolars and molars. As shown in Fig. 3, the number of patients with positive findings of these microorganisms increased from T0 to the maximum in T2, followed by decrease in T3 recording.

Table 1. **Total bacterial count compared between different recording periods on incisors, premolars and molars**

	T0–T1				T0–T2				T1–T2				T2–T3			
	Npr (*m*)	Nnr (*m*)	*Z*	*P*-value	Npr (*m*)	Nnr (*m*)	*Z*	*P*-value	Npr (*m*)	Nnr (*m*)	*Z*	*P*-value	Npr (*m*)	Nnr (*m*)	*Z*	*P*-value
i	4 (11.50)	22 (13.86)	−3.289	<0.01	1 (1.00)	25 (14.00)	−4.432	<0.01	5 (11.10)	20 (13.48)	−2.879	<0.01	19 (13.36)	7 (13.14)	−2.122	<0.05
p	7 (10.36)	23 (17.07)	−3.291	<0.01	6 (8.83)	24 (17.17)	−3.692	<0.01	9 (11.28)	20 (16.67)	−2.509	<0.05	18 (15.14)	12 (16.04)	−0.823	>0.05
m	6 (8.33)	22 (16.18)	−3.485	<0.01	2 (4.50)	28 (16.29)	−4.597	<0.01	5 (14.40)	20 (12.65)	−2.436	<0.05	16 (12.78)	11 (15.77)	−0.372	>0.05

Npr, number of positive ranks; Nnr, number of negative ranks; *Z*, Wilcoxon *Z* score; *P*, significance; *m*, mean; i, incisors; p, premolars; m, molars.

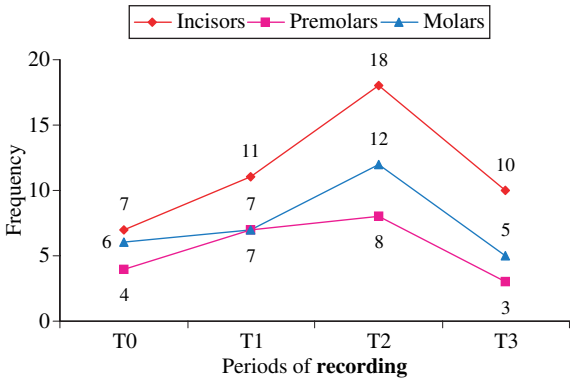

Fig. 2. Comparison of P*revotella* intermedia frequency findings on incisors, premolars and molars during periods of recording.

Table 2. **The significance of difference between positive findings of *Prevotella intermedia*. *Q*-Cochran test, *P*-level of significance**

	Statistical parameters	
	Q	*P*-value
Incisors	13.929	0.003
Premolars	8.500	0.037
Molars	9.667	0.022

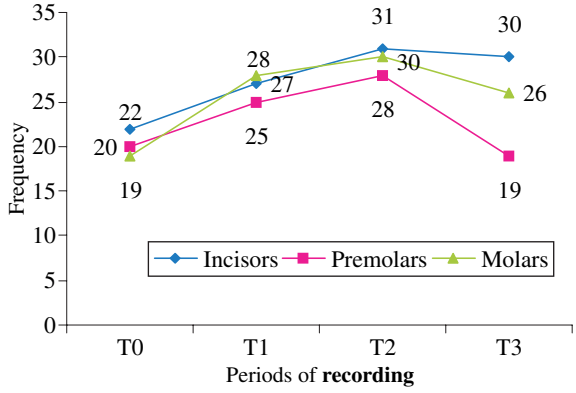

Fig. 3. Comparison of frequency findings of other pigmented anaerobic bacteria on incisors, premolars and molars during periods of recording.

Clinical parameters

During the entire study period the values of pocket probing depth were changing very similar to microbiological parameters. The measures of this clinical parameter, determined at the beginning of investigation, expressed the statistically significant tendency to increase immediately after the beginning of fixed treatment with the maximum recorded 3 months after the bonding, except for the molars. This was followed by decrease in the last T3 registration time among incisors and premolars (Table 3). Among all three investigated group of teeth the most

Table 3. **Descriptive statistical parameters of pocket probing depth at different recording periods on incisors, premolars and molars.**

	Mean				SD				CV (%)			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
i	2.500	3.039	3.211	3.188	0.386	0.436	0.550	0.557	15.440	14.347	17.129	17.472
p	1.929	2.344	2.430	2.391	0.472	0.534	0.528	0.458	24.469	22.782	21.728	19.155
m	2.461	2.805	2.914	2.930	0.512	0.503	0.419	0.361	20.805	17.932	14.379	12.321

CV, coefficient of variance; i, incisors; p, premolars; m, molars.

Table 4. **Pocket probing depth values compared between investigated teeth groups at different recording periods**

	T0				T1				T2				T3			
	d	*t*	*Z*	*P*-value	*d*	*t*	*Z*	*P*-value	*d*	*t*	*Z*	*P*-value	*d*	*t*	*Z*	*P*-value
i–p	0.570	9.240	4.630	<0.01	0.695	8.783	4.837	<0.01	0.781	9.493	4.769	<0.01	0.797	13.312	4.891	<0.01
i–m	0.039	0.492	0.599	>0.05	0.234	2.925	2.597	<0.01	0.297	3.402	2.984	<0.01	0.258	2.860	2.754	<0.01
p–m	−0.531	−6.455	4.414	<0.01	0.461	6.484	4.351	<0.01	−0.484	−5.197	3.822	<0.01	−0.539	−7.583	4.609	<0.01

d, Difference; *t*, Student *t*-test; *Z*, *Z* score; i, incisors; p, premolars; m, molars.

statistically significant values of pocket probing depth, after bonding fixed appliance to the end of the study, were measured in incisors region (Table 4).

The measurement of clinical attachment level showed very minimal values, impossible for statistical analyses and therefore could be ignored.

All the clinical measurements were performed by the same investigator at each recording in order to achieve the higher level of objectiveness. During the study duplicate examinations were conducted by examiner on five subjects using measurement of pocket probing depth and clinical attachment level to asses the method error of the used probing technique and assessment of clinical attachment level. The test showed good intra-examiner repeatability for clinical parameters reproducibility.

Discussion

The inflammatory reaction of gingival tissue can often be detected among patients wearing fixed orthodontic appliances (14, 15). The lack of adequate oral hygiene in these patients is presented as the main cause of bacterial plaque accumulation, increased number of bacterial colonies and consecutive inflammatory response. Therefore, one of the most important aims of this investigation was to determine the total bacterial count at different recording periods and its changes during fixed orthodontic treatment.

Evaluation of total bacterial count changes in subgingival dental plaque collected from incisors, premolars and molars showed the increase from the first recording (before fixed therapy) to T2 recording time. The results also show the decrease of these values 6 months after the beginning of orthodontic therapy. The maximum values of total bacteria count after 3 months of wearing fixed appliances and later decrease of this parameter correlates with the earlier reports of high resistance of periodontal tissues in young individuals (16).

However, only some specific forms of subgingival bacteria can directly deteriorate periodontal tissues (17), but this problem was less explored in previous studies. The most dangerous microorganisms that can cause periodontal damaging belong to the group of black pigmented anaerobes such as *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis* and others (12, 18). Therefore, this study was designed to evaluate the changes of periodontopathogenic bacteria during fixed orthodontic therapy. According to the results, the changes of positive findings of *P. intermedia* colonies were very dynamic. The presence of this anaerobe in subgingival plaque was detected only in a few patients before application of fixed appliances. During later treatment significant increase in number of patients with positive findings of *P. intermedia* was found with the maximum showed in T2 recording. Further, the decrease of number of patients with *P. intermedia* was found in the last T3 recording. Although the similar results appeared in all three groups of investigated teeth the most statistically significant changes in presence of this anaerobe were detected in subgingival plaque collected from incisors. These results are opposite to some of the previous reports in which most of the changes of plaque microflora were recorded around molar bands (1, 4). On the other hand, the dynamic of changes in *P. intermedia* positive findings during periods of recording correlates with the results of other authors (17, 19).

The most dangerous periodontopathogenic anaerobe is *A. actinomycetemcomitans* which causes the aggressive periodontitis (5, 20, 21). In previous studies authors found the significant increase of *A. actinomycetemcomitans* in dental plaque samples after application of fixed appliances, followed with its constant presence during the whole treatment and finally with the decrease in its findings after the removal of fixed appliance (22). However, the results of this study show that *P. intermedia*, as periodontopathic anaerobe, is more frequent in dental plaque collected from teeth with elements of fixed appliances, than *A. actinomycetemcomitans* as highly specific periodontopathic microorganism. On the other hand, despite high frequency in positive findings of *P. intermedia*, we can conclude that there are no long-term bad effects of this phenomenon on periodontal health, concerning the transition of *P. intermedia* findings during recordings and its significant decrease in the end of this study. Nevertheless, this fact does not exclude the possible risk for development of periodontal tissues damaging in some of the patients treated with fixed appliances. This confirms the opinion that, beside virulence of microorganisms, the bacterial serotype and individual host sensitivity may be the main factors for development of periodontal damaging (9).

The possible explanation for detection of *A. actinomycetemcomitans* in only one case could be the fact that this bacteria shows the major prevalence in cases of advanced lesions of periodontitis. This anaerobe expresses more increased frequency of detection in severe periodontitis than in plaque samples collected from mild periodontitis, gingivitis or healthy sites. Since the minimal values of clinical attachment level in this study point that the increase of pocket probing depth was due to gingivitis and not the result of apical movement of epithelium insertion, and the development of periodontal pockets, this could explain the presence of *A. actinomycetemcomitans* in only one patient.

The family of black-pigmented periodontopathic anaerobes consists of many other microorganisms like *P. gingivalis* and *F. nucleatum* which were also investigated in this study as the group of all other possible periodontopathogenic bacteria. Almost two-thirds of patients presented positive finding of this group of anaerobes before application of fixed appliance. After the increase in T2 recording, the most significant decrease in number of patients with positive finding of these anaerobic bacteria was detected among premolar samples in the last T3 recording.

Since the values of pocket probing depth in T2 recording period increased to the maximum it could be concluded that the presence of fixed appliance expresses its effect on periodontal health status in this short period of time starting immediately after bonding. On the other hand, the results showed clearly the tendency of decrease of this clinical parameter 6 months after the bonding which correlates with findings of Alexander (1), who reported the lack of periodontal destruction in longer period of time among patients treated with fixed appliances.

Furthermore, it must also be noted that the clinical studies detected the greater loss of clinical attachment level in the distal parts of teeth arches during fixed therapy (1). This was explained with worse oral hygiene in molar regions around bands. In this study the results are opposite. In fact, pocket probing depth expressed its maximum on incisors and it was statistically significant comparing with premolars and molars. These results prove that the brackets, same as bands, could have bad influence on periodontal health. The possible explanation for worse clinical and microbiological results on incisors could be mechanical removing of bacterial colonies during molar banding which makes their bad effect impossible. Scheie *et al.* (23) concluded that the application of fixed orthodontic appliances creates transitional decrease in the number of *S. mutans* colonies number. This phenomenon was explained with possible eliminations of bacteria colonies during banding process. Three months after the application of fixed appliance increased the number of bacteria colonies in dental plaque and saliva because fixed appliance created new retentive places. Authors emphasized that time is a very important factor necessary for bacteria colonies to regain their initial count, especially because the insertion of fixed appliance initially causes microbial elimination. This means that the mechanical removal of dental plaque caused by band placement process have an effect not only at the time of teeth banding but also during period of time after insertion of fixed appliance.

One of the interesting results of this study is connected with the measurement of clinical attachment level. These obtained values have no clinical relevance but, on the other hand, statistically significant changes of pocket probing depth, especially on incisors, are of clinical relevance because these values indicate that all reactions during fixed orthodontic therapy are the consequences of gingival reaction and not the result of deterioration of deeper periodontal tissues.

The other very important conclusion of this study is that changes in the microbial flora of subgingival dental plaque during fixed orthodontic treatment will not have long-term destructive effects on the periodontal tissues. This conclusion is based on the fact that the values of clinical and microbiological parameters start to decrease after 3 months of wearing fixed appliance and this trend continues till the end of the study. On the other hand, the evidence from the

literature also suggests that alteration of the subgingival bacterial ecosystem during orthodontic treatment does not influence the development of periodontal diseases in the long-term (2, 3).

Culturing microorganisms, the method used in this study, remains an essential and central part of microbiology. The best way to find out information about the physiology, capabilities and phylogenetic characteristics of microorganisms is to isolate and grow them in pure culture. However, current culture-based methods for studying microflora are recognized sometimes as insufficient since some microorganisms cannot be cultured under laboratory conditions. That is why new molecular DNA-based methods (denaturing gradient gel electrophoresis–DGGE and microarrays) are needed for more precise analysing of microorganisms.

After comparing the sensitivities of DGGE with those of cultivation for the detection of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* Zijnge *et al.* (24) found that the results of DGGE correlate with those of cultivation for the detection of clinically relevant periodontal pathogens. On the other hand, DGGE outcompetes cultivation in its sensitivity for the detection of *A. actinomycetemcomitans*. Comparing culture analysis with microarrays Vianna *et al.* (25) showed that one of the advantages of microarray analysis is that it can provide results within 8 h, whereas the conventional techniques involving culturing require days.

Finally, the fact is that microarrays and DGGE may provide significant additional information regarding microbiota by detecting bacterial species that are otherwise difficult or impossible to culture. The combination of all these techniques would provide the best results.

In conclusion, the results of this study confirm the fact that treatment with fixed appliances may increase the growth of pathogenic bacteria and anaerobes. These microbiological changes are strictly limited to subgingival dental plaque collected from the teeth with elements of fixed appliance and they do not have the destructive effect on periodontal tissues. Since these changes in subgingival microflora increase the risk of periodontal tissue damaging, it is necessary to provide instructions for good oral hygiene among patients undergoing fixed treatment and to maintain their constant remotivation and continual control during the whole time of orthodontic therapy.

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