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Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents

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Structured Abstract

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Objective – To determine the effects of fixed orthodontic appliances on periodontal health and microbiological composition of subgingival dental plaque.

Material and methods – This prospective longitudinal self-controlled study was conducted on 32 adolescents (13 males, 19 females), who were scheduled for fixed orthodontic treatment between 2002 and 2005. Dental plaque accumulation, gingival inflammation and pocket probing depth were measured at the mesio-vestibular angle of the examined group of teeth followed by collection of subgingival dental plaque samples in the same points. These periodontal indices and microbiological parameters were determined prior to the placement of fixed appliances and 1, 3 and 6 months after the beginning of orthodontic treatment.

Results – All values of both clinical and microbiological parameters started to increase after the placement of fixed appliances. Maximum values were reached 3 months after fixed appliance placement followed by their decrease in the last registration period of 6 months after the placement of fixed appliances.

Conclusions – Treatment with fixed appliances in adolescents may transitionally increase the values of all periodontal indices and stimulate the growth of periodontopathogenic bacteria, but without destructive effects on deep periodontal tissues.

Key words: anaerobes; brackets; dental plaque; gingival tissue; subgingival microflora

Introduction

During orthodontic therapy with fixed appliances, inflammatory reaction of gingival tissue can very often be observed. It seems that the main factor for an increased accumulation of dental plaque and inflammatory response is the appearance of new retentive places around the components of fixed appliances attached to the teeth (1). Bonding of brackets presents a major advancement in orthodontics after years of using multibanded fixed appliances. However, these changes in the design of fixed appliances did not reduce much plaque accumulation around brackets, which makes this problem persistent and very actual in fixed orthodontics (2).

Parallel to the clinical investigations of periodontal indices, such as plaque and gingival indices, and pocket probing depth, microbiological studies revealed significant changes in the bacterial composition of subgingival dental plaque (3–5). Orthodontic treatment may affect the equilibrium of oral microflora and increase bacteria retention. As a result of these changes, the inflammatory response of periodontal tissues appears, which is detected by periodontal indices measurements. The most interesting field of research presents the determination of periodontopathogenic bacteria changes in subgingival dental plaque in patients undergoing fixed orthodontic treatment (6, 7). This includes evaluation of quantitative and qualitative shifts of dangerous black-pigmented anaerobes such as *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* (8–10).

Therefore, the aim of this study was to investigate the influence of fixed orthodontic appliances on periodontal tissues, using various indices, and quantitative and qualitative changes in subgingival anaerobe microflora.

Materials and methods

Subjects

This prospective study included 32 patients of both sexes (13 males, 19 females) aged between 12 and 18 years who were patients of the Clinic of Orthodontic, Faculty of Stomatology, University of Belgrade. All recruited adolescents fulfilled the following criteria for participation in this study (11): indication for fixed orthodontic therapy regardless of malocclusion type, good general and initial periodontal health, 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.

Fixed orthodontic technique and oral hygiene

The fixed orthodontic treatment was performed with directly bonded Ricketts brackets on incisors, canines and premolars, and orthodontic bands cemented with glass-ionomer cement on the first molars. Three weeks before starting treatment, the subjects received oral hygiene instructions (tooth-brushing Bass technique) (1) and were supplied with adequate approximal and tooth brushes for fixed appliances. Instructions consisted of information about the correct use of tooth brush and interdental brush. Professional scaling and polishing were not performed to prevent the possibility of eradicating periodontal pathogen microorganisms from the subgingival environment, which would automatically change the composition of dental plaque and real results of the study.

Clinical procedures

The health status of periodontal tissues was determined by using periodontal indices according to the criteria of the plaque index system (PlI) (12), gingival index system (GI) (13) and gingival bleeding index system (GBI) (14) followed by an assessment of the pocket probing depth with a calibrated periodontal probe (Goldman-Fox, Hu-Friedy Mfg Co., Inc., Chicago, IL). The probe with accurate millimeter scale was inserted, without using force, into the deepest part of gingival sulcus until resistance appeared. The pocket probing depth was measured to the nearest millimeter on the scale. These clinical parameters were assessed on the mesio-vestibular point and in the middle point of oral tooth surface on the examined teeth: upper right first molar, upper left central incisor and first premolar, lower left first molar, lower right central incisor and first premolar. These teeth were selected according to the Rämfjord system in which each group of teeth (incisors, premolars and molars) has its representative. Between the partial recording method suggested by Ramfjord and a full-mouth recording of periodontal status exists an excellent agreement (15).

The measurements were obtained at the first patient appointment (TX), after 3 weeks before placement of the appliances (T0), and 1 (T1), 3 (T3) and 6 (T6) months after the start of orthodontic treatment. All the clinical measurements were performed by the same investigator at each control strictly respecting international criteria for the determination of periodontal indices scores (12–14). To test intra-examiner reliability for index reproducibility the examiner performed duplicate examinations on five subjects using the plaque index (PlI), gingival index (GI), gingival bleeding index system (GBI) and pocket probing depth. The test showed very good intra-examiner repeatability.

Microbiological procedures

The material for microbiological examination was obtained from mesio-vestibular points of subgingival sulcus of the following teeth: upper right first molar, upper left central incisor and upper left first premolar. If one of the representative teeth was missing, the adjacent tooth from the same group of teeth was used instead. With the exception of plaque index, which was detected first, the microbiological samples were collected prior to the other clinical procedures in four periods of time: just before the placement of fixed appliances (3 weeks after oral hygiene instruction was given), after 1, 3 and 6 months since the beginning of the orthodontic treatment. As the plaque index was always determined first, supragingival dental plaque was completely removed, which enabled solely the collection of subgingival plaque as microbiological samples. Following sampling, gingival status was assessed and the pocket probing depth was measured. The microbiological investigation was performed to confirm presence or absence of periodontopathic anaerobes: *P. intermedia*, *A. actinomycetemcomitans* and the group of other black-pigmented anaerobes such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.

The subgingival plague samples were collected in dry field conditions by inserting two sterile paper points (ISO 45) (16) carefully in 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, Faculty of Medicine, University of Belgrade for further analysis. The transport medium was prepared under anaerobe conditions during 48 h before sampling and by adding 0.5 ml paraffin oil prior to subgingival plaque collection. After suspension on Vortex for 2–3 min, 100 μl of plaque samples were plated directly and also, for more precise analysis, diluted (100 μl in 2 ml 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 at the temperature between 45 and 56°C (17). The incubation lasted 72 h under anaerobic conditions with bio Merieux gas pack system (BioMerieux, Marcy L'Etoile, France) followed by semiquantative determination of anaerobe colonies using direct counting and density comparison. Subculturing, Gram-stain and identification tests of biochemical reactions were used for accurate identification of the bacteria species. The selective subculturing was performed immediately after incubation or delayed by preserving formed bacteria colonies in BHI fluid containing 10% glycerol at the temperature of −20°C. Kanamycin–vancomycin-laked blood agar was used for the selection of *P. intermedia* and other pigmented anaerobe Gram negative bacteria. Tripticase soy-serum vancomycin bacitracin agar (TSVB) was the selective medium for isolation of *A. actinomycetemcomitans*. *P. intermedia* were identified by positive indole production, hemolysis on sheep blood and negative reaction for nitrate reduction.

Statistical analysis

Statistical analyses included descriptive statistical measures, Student's *t*-test and chi-squared test combined with McNemar test.

Results

Clinical parameters

The plaque index showed minimum values at 3 weeks after the patients were given the oral hygiene instruction, just before the placement of fixed appliances. In all three groups of examined teeth, after the attachment of bands and brackets, the values of plaque index showed an increase during the next periods with a maximum at T3 followed by a decrease at 6 months after the beginning of fixed therapy (Table 1).

All other three clinical parameters, gingival index (Table 2), gingival bleeding index (Table 3) and pocket probing depth (Table 4), showed very similar changes during the entire study period.

Microbiological parameters

Among the results obtained in microbiological phase of investigation, it could be clearly seen, that there exists a very high statistically significant difference in the number of isolated bacteria species from the collected subgingival dental plaque of the incisors, premolars and molars between pretreatment and at T3. The number of bacteria species showed its maximum

Table 1. **Statistical parameters of plaque index values**

	M	SD	CV (%)
Incisors			
Tx	1.281	0.310	24.110
T0	0.898	0.329	36.637
T1	1.211	0.278	22.956
T3	1.250	0.336	26.880
T6	1.219	0.275	22.560
Premolars			
Tx	0.883	0.298	33.749
T0	0.547	0.314	57.404
T1	0.984	0.126	12.805
T3	1.055	0.198	18.768
T6	1.031	0.123	11.930
Molars			
Tx	0.930	0.366	39.355
T0	0.625	0.354	56.640
T1	1.117	0.277	24.799
T3	1.109	0.219	19.748
T6	1.070	0.264	24.673

M, mean; SD, standard deviation; CV, coefficient of variation; Tx, first appointment; T0, 3 weeks later before fixed appliance treatment; T1, 1 month after bracket placement; T3, 3 months after bracket placement; T6, 6 months after bracket placement.

Table 2. **Statistical parameters of gingival index values**

	M	SD	CV (%)
Incisors			
Tx	0.586	0.288	49.147
T0	0.383	0.269	70.235
T1	1.148	0.310	27.003
T3	1.352	0.430	31.805
T6	1.305	0.380	29.119
Premolars			
Tx	0.680	0.450	66.176
T0	0.367	0.304	82.834
T1	0.836	0.395	47.248
T3	0.859	0.364	42.375
T6	0.813	0.348	42.804
Molars			
Tx	0.523	0.344	65.774
T0	0.352	0.297	84.375
T1	0.773	0.279	36.093
T3	0.805	0.322	40.000
T6	0.797	0.345	43.287

M, mean; SD, standard deviation; CV, coefficient of variation. For explanation of Tx etc., see Table 1.

3 months after the placement of fixed appliances (Table 5).

The number of patients with positive findings of *P. intermedia* colonies was increasing from the first control to the maximum obtained 3 months after the beginning of fixed orthodontic therapy. Six months after the placement of fixed appliances, the number of patients with isolated *P. intermedia* colonies in subgingival dental plaque decreased. 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 (Table 6).

The occurrence of other black-pigmented anaerobe bacteria showed similar changes during registration periods on examined incisors (Fig. 1), premolars (Fig. 2) and molars (Fig. 3). However, the most statistically significant changes of these bacteria were obtained among molars. As shown in Fig. 3, the number of patients with positive findings of these microorganisms reached the maximum at 3 months after the placement of fixed appliances.

Actinobacillus actinomycetemcomitans, the most potent periodontopathogenic microorganism associated

Table 3. **Statistical parameters of gingival bleeding index values**

	M	SD	CV (%)
Incisors			
Tx	0.516	0.416	80.620
T0	0.266	0.269	101.128
T1	1.320	0.586	44.394
T3	1.336	0.677	50.674
T6	1.383	0.453	32.755
Premolars			
Tx	0.320	0.366	114.375
T0	0.148	0.236	159.460
T1	0.664	0.379	57.078
T3	0.672	0.394	58.631
T6	0.594	0.415	69.865
Molars			
Tx	0.234	0.304	129.915
T0	0.227	0.249	109.692
T1	0.594	0.358	60.269
T3	0.602	0.347	57.641
T6	0.547	0.367	67.093

M, mean, SD, standard deviation; CV, coefficient of variation. For explanation of Tx etc., see Table 1.

Table 4. **Statistical parameters of pocket probing depth values (in millimeters)**

	M	SD	CV (%)
Incisors			
Tx	2.500	0.412	16.480
T0	2.500	0.386	15.440
T1	3.039	0.436	14.347
T3	3.211	0.550	17.129
T6	3.188	0.557	17.472
Premolars			
Tx	2.039	0.579	28.396
T0	1.929	0.472	24.469
T1	2.344	0.534	22.782
T3	2.430	0.528	21.728
T6	2.391	0.458	19.155
Molars			
Tx	2.531	0.549	21.691
T0	2.461	0.512	20.805
T1	2.805	0.503	17.932
T3	2.914	0.419	14.379
T6	2.930	0.361	12.321

M, mean; SD, standard deviation; CV, coefficient of variation.
For explanation of Tx etc., see Table 1

Table 5. **The difference between frequency of bacteria types number determined in different periods of control**

	McN-*p*	*p*-value
Incisors		
T0–T1	0.063	> 0.05
T0–T3	0.000	<0.01
T0–T6	0.065	> 0.05
T1–T3	0.039	<0.05
T1–T6	0.754	> 0.05
T3–T6	0.063	> 0.05
Premolars		
T0–T1	0.063	> 0.05
T0–T3	0.001	<0.01
T0–T6	0.508	> 0.05
T1–T3	0.070	> 0.05
T1–T6	0.754	> 0.05
T3–T6	0.008	<0.01
Molars		
T0–T1	0.125	<0.05
T0–T3	0.001	<0.01
T0–T6	0.092	> 0.05
T1–T3	0.039	<0.05
T1–T6	0.549	> 0.05
T3–T6	0.219	> 0.05

McN-*p*, McNemar *p*-values.
For explanation of T0 etc., see Table 1.

with juvenile periodontitis, was isolated only in one of our patients (Fig. 4). The positive findings of *A. actinomycetemcomitans* were noted on incisors at T1 and T3.

Discussion

The assessment of some selected periodontal indices (plaque index, gingival index, gingival bleeding index and pocket probing depth), measured on incisors, premolars and molars, showed an increase starting from the beginning of fixed therapy to T3 control. The results also show minimum values for all indices just before the start of orthodontic therapy. This can be explained by positive effects of oral hygiene instruction given to the patients 3 weeks before the placement of fixed appliances. As the values of all measured clinical indices increased to a maximum 3 months after fixed-appliance treatment for all three investigated groups of teeth, it could be concluded that the presence of fixed appliances expresses its influence on periodontal health in this short period of time starting immediately after the placement of bands and brackets. Therefore, the results are comparable to the results of Boyd and Baumrind (18). On the other hand, these results clearly showed the tendency of decrease of measured clinical indices 6 months after the beginning of orthodontic therapy. This phenomenon confirms the findings of Alexander (1), who reported the lack of periodontal destruction over a longer period of time among patients wearing fixed appliances.

Clinical studies on the influence of fixed appliances on the periodontal condition showed that fixed orthodontic treatment may worsen periodontal health, which improves again significantly after debonding. These studies also detected greater loss of clinical attachment level in the distal parts of the dental arches (1). This was explained with worse oral hygiene in molar regions and with the presence of bands as bigger attachments. Similar to these results, Zachrisson (19) also points to the greater stadium of gingivitis around molars, measured by periodontal indices such as PlI, GI

Table 6. **The difference between frequency findings of *P. intermedia* isolated in different periods of control**

	McN-*p*	*p*-value
Incisors		
T0–T1	0.219	> 0.05
T0–T3	0.001	<0.01
T0–T6	0.549	> 0.05
T1–T3	0.039	<0.05
T1–T6	1.000	> 0.05
T3–T6	0.021	<0.05
Premolars		
T0–T1	0.250	> 0.05
T0–T3	0.219	> 0.05
T0–T6	1.000	> 0.05
T1–T3	1.000	> 0.05
T1–T6	0.125	> 0.05
T3–T6	0.063	> 0.05
Molars		
T0–T1	1.000	> 0.05
T0–T3	0.070	> 0.05
T0–T6	1.000	> 0.05
T1–T3	0.125	> 0.05
T1–T6	0.688	> 0.05
T3–T6	0.039	<0.05

McN-*p*, McNemar *p*-values.
For explanation of T0 etc., see Table 1.

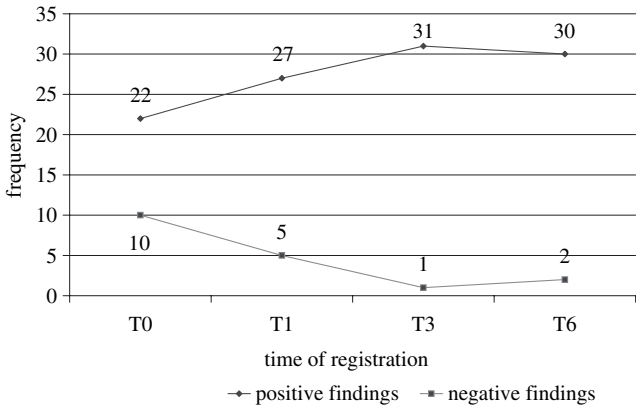

Fig. 1. Number of patients with positive/negative findings for pigmented anaerobe bacteria on incisors at different time points.

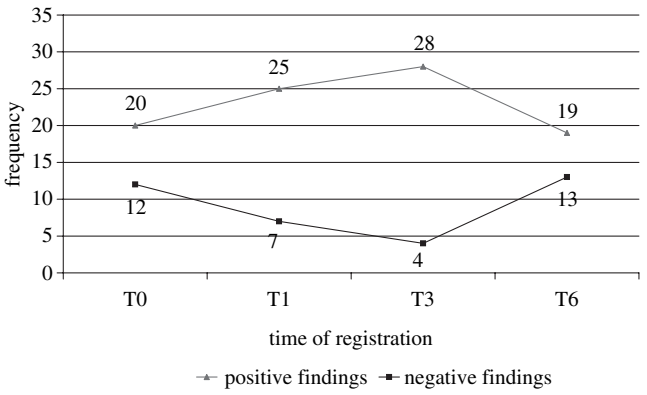

Fig. 2. Number of patients with positive/negative findings for pigmented anaerobe bacteria on premolars at different time points.

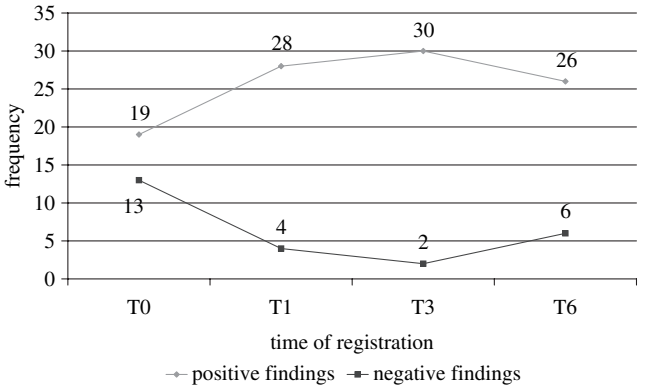

Fig. 3. Number of patients with positive/negative findings for pigmented anaerobe bacteria on molars at different time points.

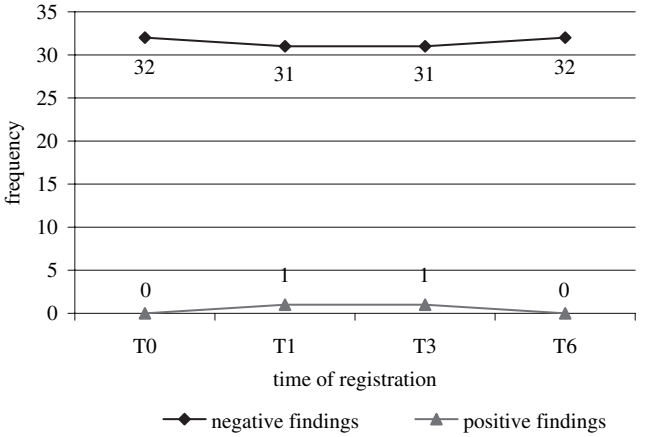

Fig. 4. Number of patients with positive/negative findings for *A. actinomycetemcomitans* on incisors at different time points.

and pocket probing depth, which could be explained with the fact that bonded attachments produce less gingival reaction compared with banded teeth. It is interesting that this study showed completely opposite results. In fact, all measured periodontal indices and pocket probing depth expressed their maximum on incisors, and it was statistically significant compared to premolars and molars. These results prove that brackets, same as bands, could have a bad influence on periodontal health.

One of the interesting results of this study is connected with the measurement of clinical attachment level. As these values were minimal for all patients included in this study and impossible for statistic analyses, it could be concluded that all reactions during fixed orthodontic therapy are the consequences of gingival reaction and not the result of deterioration of deeper periodontal tissues.

With the development of microbiology, the number of studies dedicated to specific microbiological changes in patients wearing fixed appliances increased (3, 4, 11). The efforts of researchers were aimed at very complex subgingival flora composed of periodontopathic anaerobes (6–10). Therefore, the aim of this study was to investigate the influence of fixed orthodontic appliances on qualitative changes of subgingival microflora.

Comparing the values of measured periodontal indices between the upper and lower Rämjford teeth, a high correlation was found. Based on these findings, it was concluded, that significant differences in subgingival microflora changes do not exist. For that reason, only upper teeth were tested microbiologically. The findings of other authors also confirm that highly significant correlations exist between the values of periodontal indices when they are measured from the upper jaw only or from all six Rämfjord teeth (20).

Special attention was paid to the difference between the number of bacteria types, determined in different time periods in subgingival dental plaque samples taken from incisors, premolars and molars. The number of bacteria species was given as less than six and six or more species. In all three groups of teeth, it could be seen that in the first control before orthodontic treatment, the greater number of patients had less than six bacteria species in the collected plaque samples. However, after 1 month of wearing fixed appliances, the number of patients with more than six bacteria species rose. The maximum number of patients with these results was recorded after 3 months and then followed by a significant decrease in the last control after 6 months.

However, only some specific forms of subgingival bacteria can directly deteriorate periodontal tissues (21–24). The most dangerous microorganisms, that can cause periodontal disease, belong to the group of black pigmented anaerobes such as *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis* and others (25).

According to the results, the difference in frequency findings of *P. intermedia* at different time periods is especially significant between the maximum at T3 and primary findings before orthodontic treatment. These dynamic changes in frequency of positive findings of this black-pigmented anaerobe correlate with the changes in clinical parameters measured during the experimental period. Clearly, worsening of periodontal health and inflammation is closely connected with the increased number of periodontopathic anaerobes in subgingival dental plaque.

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 a group of other potentially periodontopathic microorganisms. Their presence was detected in dental plaque samples, collected from incisors, premolars and molars, from the beginning of the investigation. Almost two-thirds of the patients presented positive findings of these anaerobes before the application of fixed appliance. This number showed an increase at later check-ups, but finally decreased at T6. The most significant decrease in the number of patients with positive findings of these bacteria was detected in premolar plaque samples.

Actinobacillus actinomycetemcomitans is claimed to be the most dangerous periodontopathogenic anaerobe and its correlation with juvenile periodontitis is documented (26, 27). In previous reports, authors found a significant increase in the positive findings of *A. actinomycetemcomitans* in dental plaque samples after application of fixed appliances followed by its constant presence during the whole treatment and finally with the decrease in its findings after the removal of fixed appliances from the teeth (28). The results of this study indicate that *P. intermedia,* as potentially periodontopathic anaerobe, is more frequent in dental plaque samples in patients undergoing fixed orthodontic treatment than *A. actinomycetemcomitans* as highly specific periodontopathic microorganism. *A. actinomycetemcomitans* was isolated only in one patient. The positive findings of *A. actinomycetemcomitans* were noted on incisors at 1 and 3 months after the placement of fixed appliances.

The results of this study can be compared with the similar studies in which clinical and microbiological effects of fixed appliances were investigated not only during the fixed treatment, but also after the removal of

bands and brackets (1). According to these studies, the values of periodontal indices and microbiological findings started to increase after the application of fixed appliances. In most of the cases the values reached their maximum after one month followed by a decrease through the entire study, especially after the removal of brackets (28). Clinical and microbiological values in this investigation were changing in similar dynamic, with the exception of reaching the maximum values 3 months after the beginning of fixed therapy.

The decrease in all clinical and microbiological parameters between M3 and M6 control, without the removal of the brackets, could be explained by reestablishment of host–microorganisms balance. It is well known that the deterioration of periodontium results from the combination of influence of different pathogen subgingival bacteria and their toxic products, and on the other hand, the response of the host and his or her defense reaction. As the attachment of fixed appliances creates new retentive places, accumulation of dental plaque and growth of subgingival pathogen bacteria increase. For this reason, the host–microorganisms balance is changed, which results in gingival inflammation and worsening of periodontal health. It seems that after about 3 months of wearing fixed appliances, the balance host–microorganisms starts to regain. This leads to a decrease in periodontopathogenic bacteria and consequently to better periodontal status. Probably, the exact mechanism of this rebalance is an increased immunodefence in gingival sulcus liquid, but further investigation in this direction is needed for an exact understanding of this rebalance process. It should also be stressed that the patients maintained good oral hygiene throughout the entire study, which also contributed host-microorganisms rebalance.

Finally, it should be emphasized once again that these results, both clinical and microbiological, are valid only for adolescents as the patients investigated in this study were at the age between 12 and 18.

Conclusions

The present results confirm the fact that, in adolescents, treatment with fixed appliances increases the values of periodontal indices and also the growth of pathogenic bacteria and anaerobes. These clinical and specific microbiological changes are strictly limited to the teeth with brackets and they do not have a destructive effect on periodontal tissues because of their transition and limitation during time. Nevertheless, as the risk of periodontal damage exists, it is necessary to provide continuous control of orthodontic patients, good oral hygiene instruction and constant remotivation during the whole period of fixed-appliance therapy.

References

1. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. *Am J Orthod Dentofacial Orthop* 1991;100:337–40.
2. Polson AM, Subtelny JD, Meitner SW, Polson AP, Sommers EW, Iker HP. Long-term periodontal status after orthodontic treatment. *Am J Orthod Dentofacial Orthop* 1988;93:51–8.
3. Sukontapatipark W, El-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod* 2001;23:475–84.
4. Jordan C, LeBlanc DJ. Influences of orthodontic appliances on oral populations of mutans Streptococci. *Oral Microbiol Immunol* 2002;17:65–71.
5. Petti S, Barbato E, Simonetti D'Arca A. Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. *New Microbiol* 1997;20:55–62.
6. Teanpaisan R, Douglas CWI, Walsh TF. Characterisation of black-pigmented anaerobes isolated from diseased and healthy periodontal sites. *J Periodont Res* 1995;30:245–51.
7. Nonnenmacher C, Mutters R, Flores de Jacoby L. Microbiological characteristics of subgingival microbiota in adult periodontitis, localized juvenile periodontitis and rapidly progressive periodontitis subjects. *Clin Microbiol Infect* 2001;7:213–7.
8. Paolantonio M, di Girolamo G, Pedrazzoli V, di Murro C, Picciani C, Catamo G et al. Occurrence of *Actinobacillus actinomycetemcomitans* in patients wearing orthodontic appliances. A cross-sectional study. *J Clin Periodontol* 1996;23:112–8.
9. Paolantonio M, Pedrazzoli V, di Murro C, di Placido G, Picciani C, Catamo G et al. Clinical significance of *Actinobacillus actinomycetemcomitans* in young individuals during orthodontic treatment. A 3-year longitudinal study. *J Clin Periodontol* 1997;24:610–7.
10. Saito K, Takahashi N, Horiuchi H, Yamada T. Effects of glucose on formation of cytotoxic end-products and proteolytic activity of *Prevotella intermedia*, *Prevotella nigrescens* and *Porphyromonas gingivalis*. *J Periodont Res* 2001;36:355–60.
11. Huser MC, Baehni PC, Lang R. Effects of orthodontic bands on microbiologic and clinical parameters. *Am J Orthod Dentofacial Orthop* 1990;97:213–8.
12. Silness J, Löe H. Periodontal disease in pregnancy II: correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121–35.
13. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol* 1967;38(Suppl):610–6.
14. Carranza FA, Newman MG. *Clinical Periodontology*, 8th edn. Philadelphia: WB.Saunders; 1996.

15. Mumghamba EGS, Pitiphat W, Matee MIN, Simon E, Merchant AT. The usefulness of using Ramfjord teeth in predicting periodontal status of a Tanzanian adult population. *J Clin Periodontol* 2004;31:16–8.
16. Hartroth B, Seyfahrt I, Conrads G. Sampling of periodontal pathogens by paper points: evaluation of basic parameters. *Oral Microbiol Immunol* 1999;14:326–30.
17. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994;5:78–111.
18. Boyd RL, Baumrind S. Periodontal considerations in the use of bands or bonds on molars in adolescents and adults. *Angle Orthod* 1992;62:117–26.
19. Zachrisson BU. A post-treatment evaluation of direct bonding in orthodontics. *Am J Orthod* 1977;71:173–89.
20. Tenovuo J, Nttonen TA. Application of a dehydrated test strip, HEMASTIX®, for the assessment of gingivitis. *J Clin Periodontol* 1978;5:206–12.
21. Diamanti-Kipioti A, Gusberti FA, Lang NP. Clinical and microbiological effects of fixed orthodontic appliances. *J Clin Periodontol* 1987;14:326–33.
22. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol* 1992; 63:322–31.
23. Turkkahraman H, Sayin MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S. Archwire ligation techniques, microbial colonization and periodontal status in orthodontically treated patients. *Angle Orthod* 2005;75:231–6.
24. Speer C, Pelz K, Hopfenmuller W, Holtgrave EA. Investigations on the influencing of the subgingival microflora in chronic periodontitis. A study in adult patients during fixed appliance therapy. *J Orofac Orthop* 2004;65:34–47.
25. Moore WEC, Moore LVH. The bacteria of periodontal diseases. *Periodontol 2000* 1994;5:66–77.
26. Russel RRB. Bacteriology of periodontal disease. *Curr Opin Dent* 1992;2:66–71.
27. Slots J, Feik D, Rams TE. *Actinobacillus actinomycetemcomitans* and *Bacteroides intermedius* in human periodontitis: age relationship and mutual association. *J Clin Periodontol* 1990;17:659–62.
28. Paolantonio M, Festa F, di Placido G, D'Attilio M, Catamo G, Piccolomini R. Site-specific subgingival colonization by *Actinobacillus actinomycetemcomitans* in orthodontic patients. *Am J Orthod Dentofacial Orthop* 1999;115:423–8.