

Evaluation of Subgingival Dental Plaque Microbiota Changes In Fixed Orthodontic Patients with Syber Green Real Time PCR

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Abstract

Introduction: One of the most common problems we confront in orthodontic therapy is periodontal diseases. Initial factor which causes these diseases is colonization of anaerobic microorganisms in subgingival plaque. Technically, local environmental changes related to orthodontic band and brackets may influence the bacterial species in periodontal plaque. However, it seems necessary to assess variations in subgingival plaque caused by orthodontic appliances. The aim of this study was to investigate changes in subgingival microbiota and clinical parameters before and after bracket placement. **Methods:** Clinical parameters including probing depth (PD), plaque index (PI), clinical attachment level (CAL), bleeding on probing (BOP) and gingival index (GI) were recorded and subgingival microbial samples were collected in 30 people aged between 13 and 25. As a control group, 15 persons getting matched as to their age and sex with no need to orthodontic treatment were opted using specific primers, SYBER Green Real-Time PCR was carried out in order to determine bacterial flora in stored samples. All mentioned procedures were reassessed in experimental group and in control group three months after band and bracket bonding. A descriptive analysis was conducted, and paired t test and Wilcoxon test were used for differences between groups ($P < 0.05$). **Results:** No changes in clinical attachment level were observed, but scores for bleeding on probing, plaque index, and gingival index increased 3 months after bracket

placement in experimental group ($P < 0.05$). *Prevotella intermedia* rose in experimental group after brackets placement compared with control group but *Actinobacillus actinomycetem comitans* and *Tanerella forsythia* proportions did increase. **Conclusions:** treatment with fixed appliances may transitionally raise the growth of periodontopathogenic bacteria and consequently result in gingival inflammatory response but without destructive effect on deep periodontal tissues.

Key words: Dental plaque, orthodontic bracket, subgingival microflora, syber green real time PCR.

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Introduction

The oral cavity is colonized by commensal microflora which is relatively stable in individuals and the composition of which is the result of a long-term relationship between the microorganisms and the action of numerous external and internal factors (1-3). These factors can lead to the colonization of important microorganism implicated in gingival inflammation and periodontal destruction (4). *Tanerella forsythia*,

porphyromonas gingivalis, actionobacillus actionomycetem comitans, and prevotella intermedia /nigrescens have been found more frequently in patients with inflammation and periodontal pockets than in healthy subjects (5). Inflammatory reaction of gingival tissue appears very often during orthodontic therapy with fixed appliances (6). The presence of new retentive places around bands and brackets is considered to be the main factor for an increased accumulation of dental plaque and inflammatory responses (7,8). Improper plaque control and undetected gingival inflammation might promote periodontal destruction that can be aggravated by orthodontic treatment.

The aim of this study was to compare the changes in subgingival microbiota and periodontal parameters before and after the placement of orthodontic brackets.

Materials and Methods

This prospective study was carried out on 30 persons (16 males and 14 females) aged 13 - 25 years. Fifteen of these subjects wore no orthodontic appliances, thus considered as control group. The other had worn fixed orthodontic appliances for at least 3 months. All of the participants in the study were recently referred to the Department of Orthodontics at the Dental School of the Mashhad University of Medical Sciences. The study design was approved by the ethics committee on human research. All subjects signed informed written consent, parents signed and approved the participation of underage patients (<18 year of age). Exclusion criteria included periodontitis, antibiotic intake in the previous 3 months, pregnancy and systemic illness. A full periodontal examination was performed by the same clinician using a marked periodontal probe (UNC-15, Hu Fridy, Chicago, III) before and 3 months after bracket placement. Ramjford teeth (3, 9, 12, 19, 25 and 28) were selected in this study.

The following parameters were recorded: probing depth (PD), plaque index (PI) (9), clinical attachment level (CAL), bleeding on probing (BOP) and gingival index (10).

These clinical parameters were assessed on the middle aspects of tooth surface on the examined teeth.

Sample collection

The samples for microbiological examination were obtained from subgingival areas of index teeth. If some of the represented vital tooth was missing, the adjacent tooth was used instead. The subgingival plaque samples were collected after appropriate tooth isolation from saliva. Visible supragingival plaque was removed and a sterile paper point was inserted into the subgingival sulcus and kept for 15 seconds before it was removed. The paper points were inserted in a microtube containing 1ml phosphate buffer (PK=8.0). Samples were placed on ice and immediately transported to the microbiology laboratory.

Lyophilized primers were used as received from the manufacturer. The sequence of primers is shown in Table 1. Following DNA extraction, Syber Green real time PCR was performed using a 5prime kit in a total volume of 25ml containing Real Master Mix Syber ROX 2.5x at 11.25ml, forward primer at 1ml, reverse primer at 1ml, template at 6ml and D.D.W at 6.75ml. Real-time PCR was run on a thermal cycler (Astech Japan) under the following conditions: primary denaturation at 95° C for 8min, denaturation at 95 °C at 20 sec, annealing at 53 °C at 20 sec and extension at 72 °C at 20 sec.

Results

Clinical parameters, plaque index, gingival index and bleeding on probing were elevated after 3 months. This elevation was significantly greater in test group than in control group (Tables 2 and 3).

The changes of three putative periodontopathogens in the subgingival plaque were detected, and are shown in Table 3. Prevotella intermedia was significantly more prevalent in the experimental group than in two other microorganisms., A. actinomycetem comitans and T. forsythia were found in subgingival plaque after 3 months but the changes were not significant as compared to baseline (P > 0.05).

Table 1. Primers for syber green real time polymerase chain reaction analyses

Bacteria name	Sequence	Product
A. actinomycetem comitans	Forward: AAA CCC ATC TCT GAG TTC TTC Reverse: ATG CCA AAC TTG ACG TTA AAT	551 nt
T. forsythia	Forward: GCG TAT GTA ACC TGC CCG CAT Reverse: TGC TTC AGT GTC AGT TAT ACC	641 nt
P. intermedia	Forward: CAA AGA TTC ATC GGT GGA Reverse: GCC GGT CCT TAT TCG AAG	294 nt

Table 2. Mean, standard deviation (S.D), minimum and maximum of changes Plaque index and gingival index

Variable	group	N	Mean±S.D	Minimum	Maximum	P-value
d.PI [†]	Experimental	15	-1.09±0.44	-1.80	-.40	<0.001
	control	15	.11±0.29	-.40	.60	
d.GI [‡]	Experimental	15	-.78±0.25	-1.50	-.40	<0.001
	control	15	.04±0.15	-.20	.40	

†: Difference between baseline and 3 month after operation for Plaque index

‡: Difference between baseline and 3 month after operation for gingival index

Table 3. The changes of Treponema Forsythia, Actinobacillus actinomycetem, Prevotella intermedia and BOP before and 3 month after operation

	Treponema Forsythia		Actinobacillus actinomycetem		Prevotella intermedia		BOP	
	No	yes	No	yes	No	yes	No	yes
Before	14	1	12	3	14	1	15	0
After	11	4	12	3	6	9	5	10
P-value	0.25		1.00		<0.001		0.002	

Discussion

The inflammatory reaction of gingival tissue can often be detected among patients wearing fixed orthodontic appliances (11,12). 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 consequent inflammatory response.

In this study, we compared the sub-gingival microbiota composition before and after the placement of brackets in patients scheduled for orthodontic treatment. It was found that brackets influenced on the composition of the sub-gingival microbiota indirectly. Clinical parameters (PI, GI and BOP) increased after orthodontic treatment, indicating that dental plaque accumulation might be the main reason for the gingival inflammation observed in these patients. Using Syber-Green real time PCR technique, we described the changes in the sub-gingival microbiota in patients receiving orthodontic treatment. Advantages of PCR technique compared to other techniques is that the technique may provide significant additional information regarding microbiote by detecting bacterial species that are otherwise difficult or impossible to culture (13) and this technique yield results in less time (14). Moreover the technique is also capable of detecting DNA from dead bacteria. Supra-gingival plaque accumulation is important because it influences the composition of the sub-gingival microbiota. After a short time (3 months) of bracket placement, the counts and frequencies of *A. actinomycetem comitans*, *P. intermedia* and *T. forsythia* increased and *P. intermedia* was significantly more prevalent in orthodontic patients. This finding suggests that orthodontic treatment might

affect the presence of periodontal microorganisms (15). The microorganisms evaluated in this study, are the most of ten implicated microorganisms as etiologic causes of periodontal damage (16).

Dramatic and significant surge in *P. intermedia* is very important finding because the increase of anaerobe microbes may be the preceding event for changes of clinical parameters. In orthodontic patients, as bacterial plaque accumulates, t inflammatory cell infiltration could increase in size and number causing the gingival tissues to swell, bleed and become edematous (17).

One of the most implicated periopatogenic anaerobe is *A. actinomycetem comitans* which causes aggressive periodontitis (18-20). In previous studies authors found a significant increase of *A.a* in dental plaque samples after application of fixed appliances, followed by constant presence during the whole treatment and finally with a decrease after the removal of fixed appliance (21).

However, the results of this study show that *P.I* as a periodontopathic anaerobe is more frequent in dental plaque collected from teeth with fixed appliances compared to *A.a*. It could be concluded that transient changes in the microbial flora of sub-gingival dental plaque during fixed orthodontic treatment will not have destructive effects on the periodontal tissues at least up to 3 months. The mere presence of a specific microorganisms does not mean that the patient has periodontal disease, but it depends on a complex bacteria-host interaction modulating the host's response leading to inflammation and future loss of attachment (22,23). From this view point, the results of present study are similar to several previous studies (14,24-26).

Periodontal conditions in patients undergoing orthodontic treatment should be monitored carefully. Removable and fixed orthodontic appliances impede correct periodontal hygiene, resulting in more plaque accumulation, inflammation and bleeding (27). Therefore, appropriate oral hygiene methods and instruments should be used to control plaque (28). Powered and interdental toothbrushes and special types of floss have been shown to improve plaque control in orthodontic patients (27,28).

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 appliances and they do not have the destructive effect on periodontal tissues. Since these changes in sub-gingival micro flora 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 re-motivation and continual control during the whole time of orthodontic therapy.

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