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Original Research Article.

Effect of Fixed Orthodontic Appliances on Subgingival Plaque Microbiota

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ABSTRACT

Introduction: The present study aimed to examine changes in subgingival microbiota and clinical parameters before and after placement of bracket.

Methods: Clinical parameters such as plaque index (PI), probing depth (PD), bleeding on probing (BOP), clinical attachment level (CAL), and gingival index (GI) were recorded. Additionally, subgingival microbial samples were collected from 30 individuals aged between 13 and 25.A total of 15 individuals as the control group were matched in terms of age and sex without needing orthodontic treatment using specific primers. Furthermore, SYBER Green Real-Time PCR was conducted to determine bacterial flora in stored samples. All the mentioned procedures were reexamined in the experimental and control groups three months after band and bracket bonding. A descriptive analysis was conducted, and paired t-test and Wilcoxon test were employed for differences between the groups (P<0.05).

Results: No changes in the level of clinical attachment were seen, but scores for plaque index, bleeding on probing and gingival index increased 3 months after placement of bracket in the experimental group (P<0.05). P. nigrescence in the experimental group increased after placement of brackets compared to the control group, but P. gingivalis and T. Denticola proportions increased.

Conclusions: Fixed orthodontic appliances may raise the growth of periodontopathogenic bacteria and consequently leads to gingival inflammation without destructive effect on deep periodontal tissues.

Keywords: Dental plaque, Orthodontic bracket, Real time PCR, Microbiological.

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INTRODUCTION

The increased accumulation of biofilm shows a high risk for adverse effects on the periodontium.[1-3] A large number of researchers have reported the presence of biofilm at the gingival margin as the most important etiologic factor in periodontal disease.[4-6] Orthodontic therapy along with poor oral hygiene can cause damage to the periodontium.[7-9] The components of fixed orthodontic appliances provides new retention areas suitable for bacterial colonization, resulting in an increase in the number of microorganisms.[10,11] Some studies have employed clinical parameters, including gingival index, plaque index, probing depth, and bone loss involving periodontal tissues— as a reference around orthodontic appliances.[12] A number of studies have reported that the placement of orthodontic appliances affects the subgingival microbial composition, increasing the prevalence of periodontopathogens such as Actinobacillus actinomycetemcomitans.[7] In this regard, Tannerella forsythia[13] have also been significantly related to gingival inflammation during orthodontic therapy. Speer et al., however, concluded that the level of periodontal pathogens decreased during the orthodontic therapy due to metal corrosion, imposing toxic effects on the microorganism.[14] Although the results are inconsistent in the scientific literature concerning a certain periodontopathogen, these earlier studies had some and[3] only a few studies used a polymerase chain reaction (PCR) method, having greater sensitivity and specificity compared to other microbiologic identification techniques such as cell culturing and the DNA probe method, particularly in detecting anaerobic bacteria. Furthermore, limited information is available on the diversity change in microorganisms according to specific sites of the dental arch.

The present study aimed to compare the changes in periodontal parameters and subgingival microbiota before and after placement of orthodontic brackets.

MATERIALS AND METHODS

This prospective study was conducted on 30 individuals (16 males and 14 females) aged between 13 and 25 years. A total of 15 of these subjects wore no orthodontic appliances, thus they were regarded as the control group. The other had worn fixed orthodontic appliances for at least 3 months. All of the study participants 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 the subjects signed informed written consent forms, and the parents signed and approved the participation of underage patients (<18 year of age).

The study exclusion criteria consisted of antibiotic intake in the previous 3 months, periodontitis, systemic illness and pregnancy. A full periodontal examination was conducted by the same clinician using a marked periodontal probe (UNC-15, Hu Fridy, Chicago, III) before and 3 months after placement of bracket. Ramjford teeth (3, 9, 12, 19, 24 and 27) were selected in the present study.

The parameters below were recorded: probing depth (PD), plaque index (PI)[15], bleeding on probing (BOP), clinical attachment level (CAL), and gingival index.[16] These clinical parameters were investigated on the middle aspects of tooth surface on the examined teeth.

Sample Collection: The microbiological examination samples were obtained from subgingival areas of the index teeth. When some of the represented vital tooth was missing, the adjacent tooth was used instead. The subgingival plaque samples were collected after isolating tooth appropriately from saliva. Visible supragingival plaque was removed, and a sterile paper point was inserted into the subgingival sulcus and maintained for 15 seconds before its removal. The paper points were inserted in a microtube with 1ml phosphate buffer (PK=8.0). The samples were placed on ice and immediately transported to the microbiology laboratory. Lyophilized primers were used as received from the manufacturer. Table 1 presents the sequence of primers. Following DNA extraction, Syber Green real time PCR was carried out using a 5prime kit in a total volume of 25ml with 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 utilized on a thermal cycler (Astech Japan) under the following conditions: primary denaturation at 95º C for 8min, denaturation at 95 ºC at 20 sec, annealing at 53 ºC at 20 sec and extension at 72 ºC at 20 sec.

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

Bacteria name	Sequence	Product
P. nigrescence	Forward: TCCACCGATGAATCTTTGGTC Reverse: ATCCAACCTTCCCTCCACTC	551 nt
P. gingivalis	Forward: TGGTTTCATGCAGCTTCTTT Reverse: TCGGCACCTTCGTAATTCTT	404 nt
T. denticola	Forward: : CCTTGAACAAAAACCGGAAA Reverse: GGGAAAAGCAGGAAGCATAA	316 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	1.1±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 P. Nigrescence, P. Gingivalis , P. Denticola and BOP before and 3 month after operation

	P. nigrescence		P. gingivalis		T. denticola		BOP	
	No	Yes	No	Yes	No	Yes	No	Yes
Before	8	7	15	0	13	2	15	0
After	1	14	13	2	8	7	10	5
P-Value	0.016		0.500		0.063		0.002	

RESULTS

Clinical parameters, including gingival index, bleeding on probing and plaque index were elevated after 3 months. This elevation was significantly greater in the test group than in the control group (Tables 2 and 3). As Table 3 shows, the changes of three putative periodontopatogens in the subgingival plaque were identified. P. Nigrescens was significantly more prevalent in the experimental group than in the two other microorganisms. T. Denticola and Porphyromonas Gingivalis were found in subgingival plaque after 3 months; however, the changes were not significant in comparison to baseline (P > 0.05), and BOP was significantly increased in the experimental group after 3 months.

DISCUSSION

The inflammatory reaction of gingival tissue in most cases can be detected in patients wearing fixed orthodontic appliances.[17,18] In these patients, the main cause of bacterial plaque accumulation, increased number of bacterial colonies and consequent inflammatory response is presented as the lack of adequate oral hygiene. In the present study, we compared the sub-gingival microbiota composition before and after placement of brackets in patients scheduled for orthodontic treatment. It was observed that brackets indirectly affected the composition of the sub-gingival microbiota. Increased clinical parameters (PI, GI and BOP) after orthodontic treatment demonstrated that dental plaque

accumulation might be the major reason for the gingival inflammation seen in these patients. We, using Syber-Green real time PCR technique, described the changes in the sub-gingival microbiota in patients receiving orthodontic treatment. The advantages of the PCR technique compared to other techniques is that the technique may present significant additional information regarding microbiote by detecting bacterial species that are otherwise difficult or impossible to culture.[19] Furthermore, the mentioned technique yields results in less time and is capable of detecting DNA from dead bacteria.[20] Accumulation of supra-gingival plaque is important, since it affects the composition of the sub-gingival microbiota. After a short time (3 months) of placement of bracket, the frequencies and counts of porphyromonas gingivalis, P.N and T. denticola increased, and P.N was significantly more prevalent in orthodontic patients. This finding demonstrates that orthodontic treatment might impact the presence of periodontal microorganisms.[21] In the present study, the microorganisms evaluated are the most of 10 implicated microorganisms as etiologic causes of periodontal destruction.[22]

Significant and dramatic surge in P.N is an extremely important finding, since the increased number of anaerobics may be the preceding event for changes in clinical parameters. As bacterial plaque accumulates in orthodontic patients, inflammatory cell infiltration could increase in number and size, causing the gingival tissues to swell, bleed and become edematous.[23]

T.denticula is one of the most implicated periopathogenic anaerobe causing chronic periodontitis.[24-26] In previous studies, authors found a significant increase of T.denticula in dental plaque samples after applying fixed appliances, even before the onset of plaque accumulation and periodontal inflammation.[27]

The study results, however, indicate that P.N as a periodontopathic anaerobe is more frequent in dental plaque collected from teeth with fixed appliances compared to porphyromonas gingivalis and T. denticola. It could be concluded that during fixed orthodontic treatment, transient changes in the microbial flora of sub-gingival dental plaque will not have destructive effects on the periodontal tissues at least up to 3 months. The mere presence of specific microorganisms does not indicate that the patient has a periodontal disease. Nevertheless, this issue depends on a complex bacteria-host interaction modulating the host's response, resulting in future loss of attachment and inflammation.[28,29] Accordingly, the present study results are similar to some previous studies.[20,30,31]

Periodontal conditions in patients undergoing orthodontic treatment should be monitored closely. Fixed and removable orthodontic appliances impede correct periodontal hygiene, leading to more plaque accumulation, bleeding and inflammation.[32] Thus, appropriate oral hygiene instruments and methods should be employed to control plaque.[33] Interdental and powered toothbrushes and special types of floss have been indicated to improve plaque control in orthodontic patients.

CONCLUSION

The present study results demonstrate that treatment with fixed appliances may stimulate the growth of pathogenic bacteria and anaerobes. The mentioned microbiological changes are highly limited to sub-gingival dental plaque obtained from the teeth with elements of fixed appliances. In addition, they do not cause any destructive effect on periodontal tissues. In this regard, it is indispensable to provide instructions for good oral hygiene in patients undergoing fixed treatment and to maintain their constant re-motivation and continual control during the whole time of orthodontic therapy, since the changes in sub-gingival micro flora increase the risk of periodontal tissue damage.

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