Effect of Fixed Orthodontic Treatment on Salivary Flow, pH and Microbial Count

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Abstract

Objectives: The present study was designed to evaluate the changes in saliva properties and oral microbial flora in patients undergoing fixed orthodontic treatment.

Materials and Methods: Two important saliva properties namely the salivary flow rate and pH as well as oral microbial flora were assessed in 30 orthodontic patients before starting fixed orthodontic treatment and after six, 12 and 18 weeks of treatment. Selective media, Sabouraud dextrose agar, Mitis salivarius agar and Rogosa agar were used for isolation of Candida albicans, Streptococcus mutans and Lactobacillus acidophilus, respectively. Statistical analysis was performed using Friedman and Dunn's tests. $P < 0.05$ was considered statistically significant.

Results: After six, 12 and 18 weeks of commencing fixed orthodontic treatment, the total colony counts of Candida albicans, Streptococcus mutans and Lactobacillus acidophilus showed a significant increase. The saliva pH decreased during the orthodontic treatment ($P < 0.05$) while the salivary flow did not change significantly.

Conclusions: Fixed orthodontic treatment causes major changes in the saliva properties. The changes in oral microflora and saliva properties show the importance of caries preventive measures during orthodontic treatment.

Keywords: Orthodontic Appliances; Saliva; Colony Count; Microbial

INTRODUCTION

Malocclusion is one of the most common dental disorders and is capable of increasing the risk of periodontal disease and dental caries [1]. Orthodontic treatment of malocclusions can often resolve them, or at least prevent their progression [2]. However, complex design of fixed orthodontic appliances can affect the oral hygiene by influencing several parameters including the saliva properties and microbial count [3]. Changes in the saliva parameters such as decrease in pH, flow rate and buffering capacity of the saliva may contribute to enamel demineralization and increase the susceptibility to dental caries [4]. Among the diverse microflora of the mouth, Streptococcus mutans is the main culprit involved in this process [5-7]. Simultaneous presence of this bacterium with

Lactobacillus acidophilus and their synergistic association increase the incidence and severity of dental caries [8,9]. Candida species have also been isolated from dental plaques and caries [10]. Atack et al, [11] were among the first to show the microbiological and periodontal changes that occur during the use of orthodontic appliances. In some other studies, the effects of using these appliances on the oral hygiene were characterized [12,13]. However, some controversial results have been achieved. Therefore, the aim of this study was to assess the flow rate, pH and microbial count of the saliva after placement of fixed orthodontic appliances.

MATERIALS AND METHODS

In this observational longitudinal study, we selected subjects using non-randomized conve-

nience sampling. Thirty patients (six males and 24 females, aged 12 to 18 years) ready to commence fixed orthodontic treatment were included in this study after signing written informed consent forms. Sample size was determined based on $\alpha=0.05$ and taking similar published papers in consideration [13-16]. Among the thirty patients participating in this study, 19 patients had class I malocclusion while the remaining 11 patients had class II malocclusion. None of the patients had severe skeletal malocclusion in anteroposterior, vertical or transverse dimensions. All treatment plans were nonsurgical orthodontic treatments including non-extraction, extraction of two upper premolars, and extraction of four premolars. The inclusion criteria were good general health and oral health (absence of oral lesions, dental caries, missing teeth and periodontal disease), and not taking antibiotics. All patients were non-smokers.

Fixed orthodontic treatment

Straight wire 0.022 inch-slot brackets (American orthodontics, Sheboygan, WI, USA) were selected for this study. The same wire sequences were used for all patients and bonding process was performed with the same adhesive for all patients (Transbond XT, 3M Unitek, Monrovia, CA, USA). Treatment was started with NiTi 0.0014-inch wire and continued with NiTi 0.0016-inch, NiTi 0.0018-inch and NiTi 0.0019×0.0025-inch wire after six (T6), 12 (T12) and 18 (T18) weeks, respectively.

Salivary flow rate measurement

Salivary flow rate was assessed at four different time points: Two weeks before the start of orthodontic treatment (T1) and six weeks, 12 weeks and 18 weeks after treatment. Professional oral hygiene instruction was provided for all patients by a well-trained expert oral hygienist before starting the study. Patients were asked to brush their teeth early in the morning of their orthodontic appointment and not to eat, drink or perform any oral hygiene procedure for at least

two hours before saliva collection. Therefore, possible changes in the salivary composition were minimized. Saliva was collected between 10 and 12 a.m. The whole saliva was collected by spitting into a sterile test tube for 10 minutes. Thereafter, the volume of the saliva (milliliters per minute) collected during this period was measured and divided by 10 to attain the flow rate in millimeters per minute [14].

Saliva pH measurement

The pH values were measured with portable pH meter strips at the same four time points.

Microbiological procedures

For isolation of Candida species, 0.1 mm of the collected saliva was cultured in a selective medium (Sabouraud dextrose agar + chloramphenicol) for 48 hours at 37°C. The number of colony forming units per milliliter (CFU/ml) was then quantified using the manufacturer's instructions and the related charts by an expert microbiologist. Mitis salivarius agar medium with bacitracin and sucrose supplement was used for isolation of Streptococcus mutans. Hydrolysis of hippurate and catalase test were also applied for identification of Streptococcus mutans. Isolation of Lactobacillus acidophilus was performed in a Rogosa selective medium.

The Rogosa plates were then incubated for 72 hours at 37°C in anaerobic condition (85% nitrogen, 5% carbon dioxide and 10% hydrogen peroxide).

Statistical analysis was performed using the Friedman and Dunn's tests. $P<0.05$ was considered statistically significant. The data were analyzed using SPSS version 18 Software (SPSS Inc., Chicago, IL, USA).

RESULTS

Saliva flow rate

A minimal increase in the whole saliva flow rate was observed in the study period, which was not statistically significant (Table 1).

The pH value measurement

As shown in Table 2, pH value decreased from

Table 1: Saliva flow rate in fixed orthodontic treatment at four time points

Time (weeks)	Saliva flow rate (mL/min)	P-value
0	1.13±0.42	-
6	1.14±0.25	P=0.90
12	1.20±0.33	P=0.47
18	1.22±0.42	P=0.41

Table 2: The pH values of whole saliva after six, 12 and 18 weeks of treatment

Time (weeks)	pH	P-value
0	7.18±0.35	-
6	6.78±0.23	P<0.001
12	6.76±0.28	P<0.001
18	6.81±0.31	P<0.001

7.18±0.35 in T1 to 6.81±0.31 in T18. This decrease in pH value was statistically significant (P<0.001).

Microbial count

The results of Friedman test revealed statistically significant differences between the four time points (P<0.001). Therefore, the Dunn's test was used for pairwise comparisons and showed statistically significant differences in all comparisons (P<0.001). Total Streptococcus mutans and Lactobacillus acidophilus counts showed a similar increase at six weeks after the beginning of orthodontic treatment and continued up to 12 weeks. The colony count of the mentioned bacteria decreased from this point on; however, the changes were not statistically significant (Tables 3 and 4). As indicated in Table 5, the number of Candida albicans colonies also increased during the study period (P<0.001).

DISCUSSION

Placement of fixed orthodontic appliances compromises the patients' oral hygiene not only by impeding oral hygiene procedures, but also by changing the saliva properties and microbial count. Therefore, in the present study, total microbial count and salivary flow and pH were analyzed at different time points before and during fixed orthodontic treatment. In the current

study, the increase in the saliva flow rate was not statistically significant (Table 1).

This observation was almost comparable to those reported by some other authors [14,15]. However, Peros et al, [16] found that salivary flow rate increased significantly after 12 and 18 weeks of fixed orthodontic treatment. But it should be mentioned that stimulated saliva was evaluated in their study. Similar results were reported by Forsberg et al, [17]. They showed that salivary flow rate significantly increased during orthodontic therapy. Their findings were not in accordance with the result of our study, which may be due to their smaller sample size.

The salivary flow rate as an important saliva property plays a critical role in oral health. An increase in flow rate promotes the physical cleansing action of the saliva, increases its anti-microbial activities, and accelerates clearance of substrates. On the other hand, low saliva secretion rate adversely affects oral health [18].

The change in salivary flow rate as mentioned above can be considered as a physiological response to presence of fixed orthodontic appliances. The introduction of these appliances into the oral environment appears to alter the oral homeostasis. Evaluation of pH, another important saliva parameter showed a significant decrease after the beginning of orthodontic

Table 3: The effect of fixed orthodontic treatment on salivary mutans streptococci count after six, 12 and 18 weeks

Time (weeks)	Total Streptococcus mutans count CFU/mL			Mean rank	P-value
	0 (undetectable)	1 (<10^5)	2 (>10^5)		
0	1	25	4	1.93	-
6	0	17	13	2.60	P<0.001
12	0	14	16	2.80	P<0.001
18	0	16	14	2.67	P<0.001

Table 4: The effect of fixed orthodontic treatment on Lactobacillus acidophilus count after six, 12 and 18 weeks

Time (weeks)	Total Lactobacillus acidophilus count CFU/mL			Mean	P-value
	0 (undetectable)	1 ($<10^5$)	2 ($>10^5$)		
0	7	23	0	1.72	-
6	0	22	8	2.72	P<0.001
12	0	20	10	2.85	P<0.001
18	0	22	8	2.72	P<0.001

therapy (Table 2).

This result was also consistent with that of previous studies, which indicated a decrease in pH following the placement of fixed appliances [14]. Another notable finding in our study was the increase in number of Streptococcus mutans and Lactobacillus acidophilus during the study period (Tables 3 and 4).

The presence of fixed orthodontic appliances on teeth might be the likely reason for this observation. Based on the study performed by Kanaya et al, [19], the main bacterial isolates identified during orthodontic therapy were mutans Streptococci. In another study, it was indicated that Streptococcus mutans was strongly implicated in the initiation and progression of dental caries, and its presence was significantly associated with dental plaque [18].

Recently, it was also shown that during orthodontic procedures, Mutans streptococci are among the most important causes of bacterial endocarditis [20]. Although we assessed the bacterial count of the saliva for up to 12 weeks after commencing orthodontic treatment, the microbial changes might show different trends over longer treatment periods, especially based

Table 5: The effect of fixed orthodontic treatment on Candida albicans count after six, 12 and 18 weeks

Time (weeks)	Total Candida albicans count CFU/mL	P-value
0	29.78±20.34	-
6	55.19±21.27	P<0.001
12	58.41±18.31	P<0.001
18	61.14±21.17	P<0.001

on the type of orthodontic appliances.

Therefore, using in-office bacterial tests for microbial monitoring during orthodontic treatment has been suggested by Mummolo et al, [21]. It also seems logical to conduct studies to assess the saliva properties during the entire course of orthodontic treatment. In the present study, there was also a significant change in the total count of Candida species. Our results showed that among the isolated Candida species, Candida albicans was the most predominant type. As described by previous studies, this change may be due to the foreign objects like appliances, which could alter the oral microbial populations [10].

CONCLUSION

The results of this study confirmed the fact that treatment of patients with fixed orthodontic appliances may change the oral microbial flora and the saliva properties as the total colony counts of Candida albicans, Streptococcus mutans and Lactobacillus acidophilus increased and the saliva pH decreased during the course of treatment.

Due to these changes, it is recommended to provide patients undergoing fixed orthodontic treatment with more precise oral hygiene instructions.

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