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Association between clinical and salivary microbial parameters during orthodontic treatment with removable appliances with or without use of fluoride mouth rinse

ABSTRACT

Aim *To evaluate the correlation between clinical and salivary microbial parameters during treatment with removable orthodontic appliances with or without use of fluoride mouth rinse.*

Material and methods *A group of 48 patients completed this randomized, controlled, parallel-group, clinical pilot study. 24 patients of the test group (TG) rinsed after tooth brushing with a fluoride mouth rinse (100 ppm AmF/150 ppm NaF) while 21 patients of the control group (CG) did not. Clinical parameters [Approximal Plaque Index (API), Papillary Bleeding Index (PBI)] as well as levels of salivary mutans streptococci (MS) and lactobacilli (LB) were assessed at baseline and after 3, 6, 9 and 12 months. Compliance was documented by participants. Appliance wear-times were estimated retrospectively by the patients. All participants received the same brushing instructions and were supplied with the same toothbrush/toothpaste.*

Results *A significant correlation of API values with MS levels (p=0.0003) or with LB levels (p=0.001) was observed. Furthermore, appliance wear-times were significantly associated with API values (p=0.02). The changes of MS or LB levels between beginning and end of the study did not differ significantly between study groups. The changes of the API scores showed slightly different median values (-3.5 in the TG vs. 0 in the CG), the difference, however, was not significant.*

Conclusions *The results emphasise the need for a careful monitoring of the oral hygiene status in patients with removable orthodontic appliances. Larger controlled clinical trials are necessary to investigate treatment options such as use of supplementary fluoride mouth rinse.*

Keywords *Appliance wear times; Clinical study; Fluoride mouth rinse; Removable orthodontic appliances; Salivary mutans streptococci and lactobacilli.*

Introduction

Orthodontic treatment can induce oral ecological changes, which might lead to increased proportions and absolute numbers of mutans streptococci [Topaloglu-Ak et al., 2011]. This is particularly important because these changes could foster initial caries development during orthodontic therapy. So far, this issue has mainly been investigated for fixed orthodontic appliances [Boersma et al., 2005]. However, removable orthodontic appliances (ROA) could have an impact on the intraoral microbial situation as well [Batoni et al., 2001]. Unfortunately, studies on oral hygiene of patients with ROA are very limited [Topaloglu-Ak et al., 2011]. If ROA are worn between 13 and 16 hours per day - as usually prescribed - they could also exacerbate the retention of plaque and influence intraoral microbial ecology. Therefore, preventive efforts in such risk groups have focused on a regular supply of bioavailable fluoride [Benson et al., 2004].

The aim of this study was to evaluate the association between clinical and salivary microbial parameters in patients with removable orthodontic appliances. Secondary objectives were to investigate the relationship of appliance wear-times with these clinical and microbial parameters and to explore whether the use of a fluoride mouth rinse (AmF/NaF) in addition to a standard oral hygiene regime with toothpaste (1,400 ppm AmF) might help patients with removable orthodontic appliances in maintaining proper oral health.

Materials and methods

The study protocol was approved by the Ethics Committee of the hospital of Jena, approval no. 1706-01/06. The study conformed to the Declaration of Helsinki and was performed according to the guidelines of Good Clinical Practice. Before participation, all the participants and their parents/guardian(s) received full

oral and written information on the aims of the study and signed a written form of consent. Adolescent patients with removable orthodontic appliances treated at the orthodontic department of the hospital of Jena were consecutively enrolled in this 12-month, blinded, 2-arm, parallel-group randomised clinical pilot study. Patients carried mostly upper/lower removable plates; only 3 patients had functional appliances. The clinical trial consisted of three consecutive operative stages: first a screening, subsequently a baseline determination followed by a 12-month observer-blind evaluation period. At the screening appointment it was assessed whether the following inclusion criteria were fulfilled: 1) current treatment with ROA subject to a continuation of at least 12 months; 2) no history of medical problems; 3) no current antibiotic therapy; and 4) no active untreated carious lesions as assessed in an intraoral examination by a dentist. Subjects were subsequently randomly assigned to a test group (TG) or a control group (CG) using a prepared list based on random numbers. Subjects in the TG were asked to rinse their mouth vigorously, after brushing their teeth, twice daily for 30 seconds with 10 ml of an AmF/NaF fluoride mouth rinse. Subjects in the CG received the same instruction, i.e. to brush their teeth twice daily, except that they were not asked to use a fluoride mouth rinse. During the evaluation period, clinical and microbial parameters were recorded.

Clinical and microbial parameters

Oral hygiene was assessed at baseline and after 3, 6, 9 and 12 months using the Approximal Plaque Index (API) [Lange et al., 1977] and Papillary Bleeding Index (PBI) [Saxer und Mühlemann, 1975]. The PBI was determined buccally in the 1st and 3rd quadrants and orally in the 2nd and 4th quadrants by using a periodontal probe running gently along the gingival margin of the mesial and distal part of the papillae. After approximately 30 seconds, presence (+) or absence (-) of bleeding was recorded. The PBI was calculated by dividing the bleeding number by the total number of papillae examined multiplied by 100 to express the index as a percentage.

The API was determined orally in the 1st and 3rd quadrants and bucally in the 2nd and 4th quadrants. The presence (+) or absence (-) of proximal plaque was assessed using a periodontal probe and the percentage of surfaces with plaque was counted.

Initial caries lesions (IL) were recorded for each patient at the screening examination and at the end of the study. IL was defined as enamel area which clinically appeared as milky white or opaque in color. The presence (+) or absence (-) of IL was assessed separately for all smooth and occlusal surfaces of permanent teeth. The microbial parameters comprised levels of salivary mutans streptococci (MS) and lactobacilli (LB), and were assessed at baseline and after 3, 6, 9 and 12 months using the CRT® bacteria test (Ivoclar Vivadent, Schaan, Principado de Liechtenstein). The numbers of colony forming units (CFUs) of MS per millilitre were categorised as follows: low: $<10^5$ CFU/ml (scores MS 0+1); high:$\geq 10^5$ CFU/ml (scores MS 2+3). The scores used for classification of LB were similarly categorised in low: $<10^5$ CFU/ml (score LB 1+2) and high: $\geq 10^5$ CFU/ml (scores LB 3+4). Data were collected by two separate, blinded investigators. Both investigators were calibrated prior to the study.

Oral hygiene instructions and compliance

After baseline examination, all participants received oral and written tooth brushing instructions based on the Bass technique. A brushing time of three minutes was recommended. All patients were instructed to brush their teeth and their orthodontic appliances twice daily (after breakfast and before bedtime). During the study period, the subjects received the same toothbrush and identical toothpaste (elmex InterX Kurzkopfzahnbürste, elmex 1400 ppm AmF, GABA, Lörrach, Germany). The subjects of the TG were also supplied with the fluoride mouth rinse (100 ppm AmF/150 ppm NaF, GABA, Lörrach, Germany). The oral hygiene of the participants during the home-care regimen was monitored by the use of compliance diaries. The subjects of both groups were asked to record the tooth brushing performances; patients in the TG were additionally asked to indicate the mouth rinsing on a tabular rinsing diary. At the end of the study, all subjects completed a questionnaire designed to estimate, retrospectively, the appliance wear-times. Appliance wear-time was categorised as follows: fewer or more than 16 hours per day.

Statistical methods

Continuous variables are described by median and interquartile range (IRQ), binary data by absolute and relative frequencies. To describe the interrater reliability with respect to the rating of MS- and LB-scores, the Cohen´s kappa coefficient was calculated. In order to evaluate the correlations between clinical scores and categorised microbial parameters as well as categorised wear-time of the appliances, multivariable linear models with clinical scores as dependent variables were used. The correlations between categorised microbial parameters and appliance wear-time were evaluated within multivariable logistic models. The general estimating equations method [Zeger et al., 1988] was used in all models to account for repeated measurements within each participant during the course of the study. The simplified question of a correlation between the categorised MS-values (low, high) and the API score at baseline served as a scenario for a rough sample size estimation in this pilot study. Assuming a difference in the mean API-Score of 15 between the groups of patients with low or high MS-values, an equal standard deviation in both (equally sized) groups of 18, two-sided $\alpha = 0.05$ and a power of 80%, the total sample size for an unpaired T-test amounted to 48 patients. Treatment group comparison followed the intention-to-treat approach using the Mann-Whitney U-test or Fisher's exact test. Additional exploratory two-

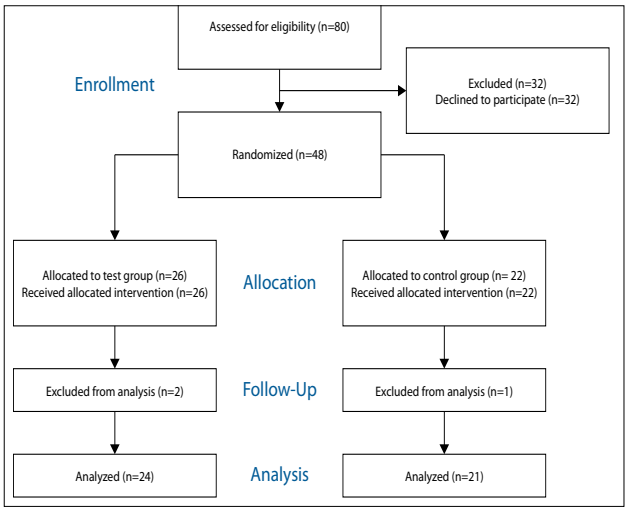

FIG. 1 Pathway of participants.

factorial non-parametric analyses of variance were carried out to adjust the group comparisons for unequal gender distribution. Results with a two-sided p-value <0.05 are regarded as statistically significant. Due to the explorative nature of the study, the p-values cannot be interpreted as error probabilities in a confirmatory sense. All statistical analyses were carried out using the Statistical Analysis System Version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

A Consolidated Standards of Reporting Trials (CONSORT) flowchart for the participants is shown in Figure 1. A total of 80 patients were examined at the screening stage. Of these, 32 patients refused to participate in the study. Finally, a total of 48 patients (29 males and 19 females) were enrolled in the study. Random assignment resulted in 26 patients in the TG and 22 in the CG. During the course of the study, two patients from the TG and one patient from the CG dropped out. The data from 24 subjects of the TG (median age: 12.5, IQR: 10-16.5; 18 males; 6 females) and 21 patients of the CG (median age: 13.0, IQR: 10.0-14.0; 9 males; 12 females) were included in the analysis. The interrater reliability for the raters was found to be Kappa = 0.96 for MS, and Kappa = 0.97 for LB indicating an excellent degree of reliability. The baseline values of clinical and microbial parameters are given in Table 1 and 2. All parameters except gender showed comparable distributions in both groups (difference in male/female ratio: p=0.028). The high plaque values and also the high levels of MS documented, at baseline, an increased bacterial caries risk for both groups.

Compliance with brushing and rinsing

The brushing diary was not available in the case of three participants, who were excluded from this evaluation. With the remaining 42 participants compliance in the documentation of tooth brushing was higher in the TG compared to the CG. Counting the missing values as "not cleaned" resulted in a different median compliance rate of TG 96.3% (IQR: 82.9-99.1%) and CG 86.2% (IQR: 73.8-97.3%) (p=0.38). The median compliance rate for rinsing was 89.3% (IQR: 73.3-96.0%) counting missing values as "not rinsed". The morning and evening compliance rates for brushing and rinsing differed only marginally, therefore only evening rates are reported here.

Compliance with appliance wear-times

The questionnaire for evaluating appliance wear-times was not available for seven patients who had to be excluded from this evaluation. The number of participants wearing the appliances longer than 16 hours was 11 (50%) in the TG and 10 (62.5%) in the CG (p=0.44).

Associations between clinical and microbial parameters

A significant positive association between API values and MS levels was observed (p=0.0003). As seen in Figure 2, the association of API values with MS was present at all examination points. Furthermore, API values showed a significant association with LB levels (p=0.001). Moreover, a significant association between PBI values and MS was observed (p=0.0006). The association between PBI values and LB was less obvious (p=0.14).

Association between appliance wear-times and clinical or microbial parameters

A significant association between appliance wear-times and API values could be detected (p=0.02). The difference in the distribution of the API scores between the groups of participants who wore the appliances for

Parameters		Test group	Control group	p value*
		n=24 subjects	n=21 subjects	
API (%)	Baseline	67.5 (52.0-85.5)	67.0 (54.0-83.0)	0.70
	12 Months	71.5 (51.0-78.0)	77.0 (54.0-87.0)	
	Change	-3.5 (-15.0-7.5)	0.0 (-7.0-10.0)	0.26
PBI (%)	Baseline	20.0 (14.0-31.0)	25.0 (16.0-29.0)	0.52
	12 Months	14.0 (7.5-23.0)	17.0 (7.0-29.0)	
	Change	-6.0 (-14.0-1.5)	-7.0 (-12.0-1.0)	0.90
*Mann-Whitney U-test, Chi-square test				

TABLE 1 Baseline values of clinical oral hygiene scores (median; interquartile range) parameters in test and control group and changes in the test and control group from baseline to the end of the study (median, interquartile range).

more than 16 hours or less than 16 hours per day can be noted at all examination times (Fig. 3). No clear association between appliance wear-times and PBI could be perceived (p=0.23). Moreover, a significant correlation of appliance wear-times with LB was observed (p=0.003) (Fig. 4). The association between appliance wear-times and MS was less pronounced (p=0.11).

Treatment comparison

The change in the API score from baseline to the end of the study displayed slightly different median values (-3.5 in the TG vs. 0 in the CG); the difference, however, was not significant (Table 1). As shown in Figure 5, the group difference in API levels is present at all examination times; however, no strict time trend within each group can be observed. During observation time, the median PBI values dropped slightly, with no significant differences between the treatment groups. Adjusting for gender did not qualitatively change the results of the treatment comparison. There were also no significant differences between the test group and control group regarding the change in the number of initial caries lesions. However, from baseline to the end of the study, the number of IL only decreased in patients within the test group [in five patients (20.8%) represented by the green lines in Figure 6]. On the other hand, an increased number of IL was only found in patients within the control group [in two patients (9.5%) represented by the red lines in Figure 6]. The distributions of levels of MS and LB at baseline and at the end of the treatment period are given in Table 2. The distributions of changes between different categories of MS or LB, respectively, during the observation period do not differ significantly between treatment groups.

Parameters		Level	Test group	Control group	p value*
			n=24 subjects	n=21 subjects	
MS levels	Baseline	low	9 (37.5)	4 (19.1)	
		high	15 (62.5)	17 (80.9)	0.17
	12 Months	low	14 (58.3)	7 (33.3)	
		high	10 (41.7)	14 (66.7)	
	Change	high to low	6 (25.0)	4 (19.1)	
		equal	17 (70.8)	16 (76.2)	
		low to high	1 (4.2)	1 (4.8)	0.86
LB levels	Baseline	low	14 (58.3)	13 (61.9)	
		high	10 (41.7)	8 (38.1)	0.81
	12 Months	low	15 (62.5)	11 (52.4)	
		high	9 (37.5)	10 (47.6)	
	Change	high to low	6 (25.0)	2 (9.5)	
		equal	13 (54.2)	15 (71.4)	
		low to high	5 (20.8)	4 (19.1)	0.37
*Fisher's exact test					

TABLE 2 Levels (low: <10^5 CFU/ml; high: ≥10^5 CFU/ml) of salivary mutans streptococci (MS) and lactobacilli (LB) at baseline and at 12 months, as well as their change in test and control group (percentage in parenthesis).

Discussion

The present study evaluated the levels of salivary mutans streptococci and lactobacilli as well as the plaque and gingival index scores in patients during 12 months of treatment with removable orthodontic appliances and provides new information on both the association between clinical and salivary microbial parameters and the impact of appliance wear-times on clinical as well as microbial parameters. Furthermore, to the best of our knowledge, this pilot study investigated for the first time whether the use of a fluoride mouth rinse, in addition to a standard oral hygiene regime, might help patients with removable orthodontic appliances in maintaining proper oral health.

Clinical and microbial parameters in patients with removable appliances

The connection between the use of removable

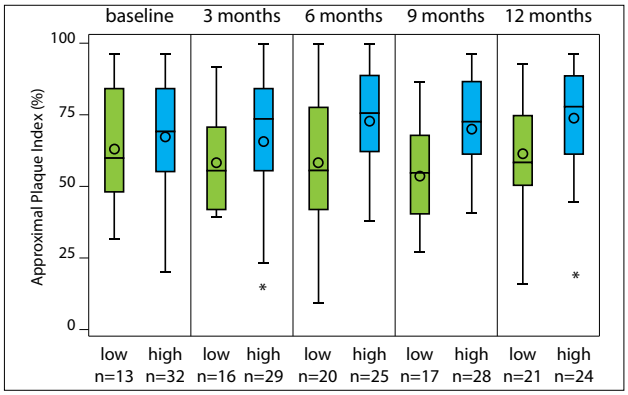

FIG. 2 Association between levels of salivary mutans streptococci (low/high: </≥ 10^5 CFU/ml) and Approximal Plaque Index during the study (p=0.0003) (n=45 subjects).

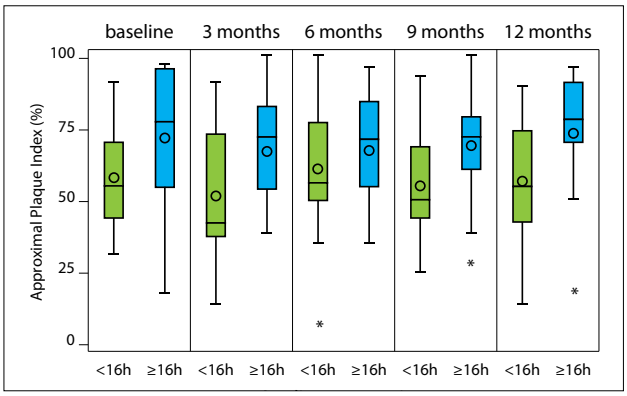

FIG. 3 Association of appliance wear-time (<16 hours versus ≥ 16 hours per day; n=17 versus 21 subjects) with approximal plaque index during the study (p=0.02).

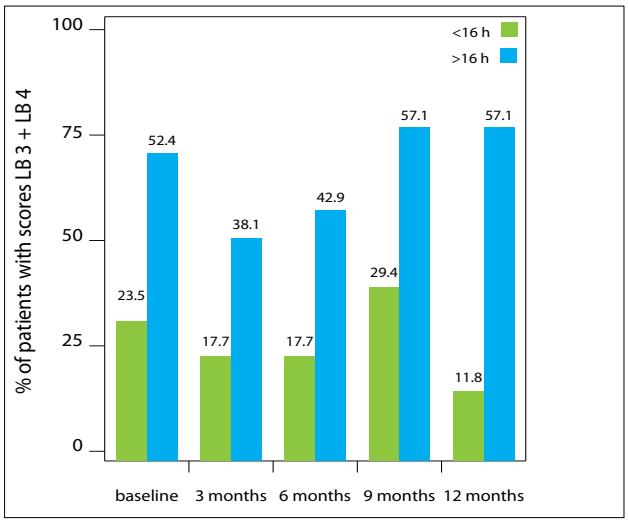

FIG. 4 Association between levels of salivary lactobacilli (LB, percentage of patients with high: ≥10^5 CFU/ml levels) and appliance wear-times (<16 hours versus ≥ 16 hours per day) during the study (p=0.003).

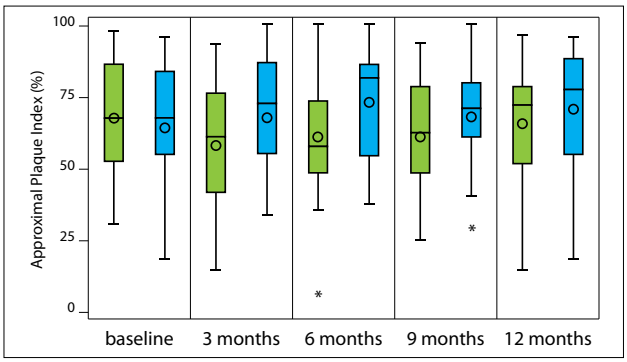

FIG. 5 API of the test [green] (n=24 subjects) and control group [glue] (n=21 subjects) during the investigation.

orthodontic appliances and the risk of caries development is still under discussion [Batoni et al., 2001]. Although various factors determine caries risk, findings higher than 10^5 CFU of salivary MS and/or LB per millilitre seem to indicate an increased risk [Krasse, 1988]. Kneist et al. [2012] compared the caries incidence of children with either low (<10^5 CFU) or high (≥10^5 CFU) counts of MS. Those with low caries risk had developed one new decayed surface within 4 years; children with high caries risk developed four within the same time period. The analysis of our data revealed that caries risk with regard to the number of MS (≥10^5 CFU) was quite high and it affected 62.5% of patients of the TG and 80.9% of the patients in the CG at the beginning of the study. High counts of MS in plaque/saliva were also found in previous studies [Batoni et al., 2001; Antoszewska et al., 2010]. In the present report, the increased numbers of MS were documented longitudinally, over 12 months of observation time. As used in previous studies we used a chairside test to estimate the counts of salivary MS and LB. Previous studies have shown that the method correlates well with conventional laboratory methods [Karjalainen et al., 2004; Tanabe et al., 2006].

Plaque control is one of the most important factors that limit the establishment and colonising of cariogenic microorganisms. Addy et al. [1982] demonstrated that the microbial plaque composition was significantly altered in removable appliance wearers if compared with non-appliance wearers as a result of increases in palatal plaque scores. Nearly all subjects in the present study population exhibited high plaque values at the beginning and throughout the observation period (Table 1, Fig. 4). In accordance with Batoni et al. [2001], only patients under current treatment with removable orthodontic appliances were included in the study, in order to guarantee high counts of mutans streptococci. This also explains the high API scores. In accordance with Antoszewska et al. [2010], these findings might indicate that the appliance material enhances dental plaque deposition, as well as stress the need for preventive strategies during the treatment period with removable appliances, such as motivating patients

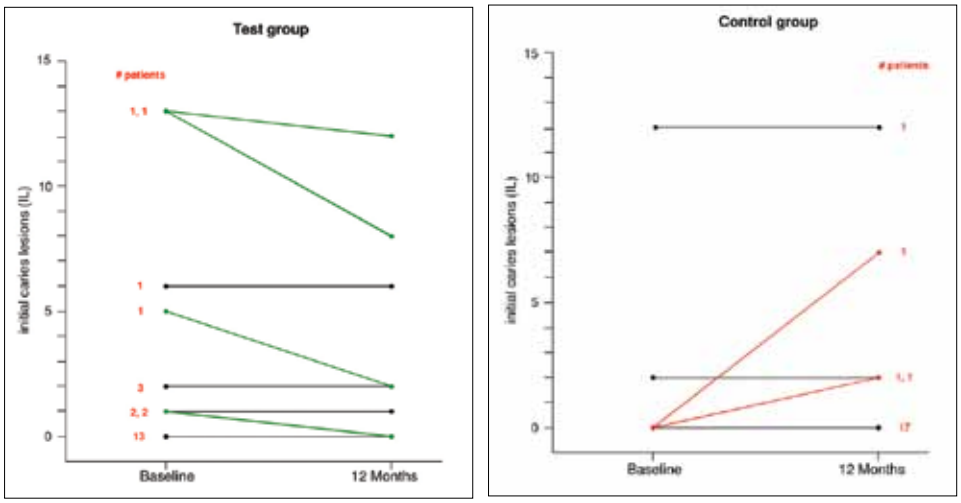

FIG. 6A, 6B Numbers of initial caries lesions (IL) in the test (left) and control (right) group and changes in the test and control group from baseline to the end of the study.

to improve mechanical plaque control and additionally use fluoridated toothpastes and mouth rinses.

Association between clinical and microbial parameters and impact of appliance wear-times

In the present study, a positive association was found between the levels of salivary MS/LB and API values as well as MS counts and PBI values. These results were in accordance with those of Stösser et al. [2000] in children with a high caries risk and correlate well with those reported by Antoszewska et al. [2010]. In contrast to the present findings Schlagenhauf et al. [1989] found no association between the quantity of plaque and the amount of caries risk-related salivary bacteria in patients wearing orthodontic appliances. Compliance with wear-time is essential for success of orthodontic treatment with removable appliances. The results of the present study suggest that the daily wearing time seems to affect the levels of salivary MS and LB and API values. In particular, the unrestricted increase of the LB, and especially of those resistant to fluoride, could promote inflammatory reactions if oral hygiene remains insufficient [Kneist et al., 2010].

The effect of a fluoride mouth rinse in addition to fluoride toothpastes on oral health

The benefits of using fluoride-containing toothpastes are firmly established and there is some evidence that a daily NaF mouth rinse reduces the severity of demineralisation associated with fixed orthodontic appliances [Benson et al., 2004]. The regular use of a mouth rinse requires a high level of patient cooperation. Therefore, patient compliance relating to home tooth brushing and mouth rinsing was monitored by means of daily compliance diaries as used in the majority of oral hygiene/tooth brushing studies [McCracken et al., 2002]. In the study by Yoshihara et al. [2001], compliance was assessed by merely interviewing the patients retrospectively. McCracken et al. [2002] reported that not always detailed analyses of the data acquired from such diaries are presented.

In the present study, median compliance rate was higher in the TG by about 10 percentage points compared to the CG. Therefore, the slight positive effect on API values (Table 1) observed in the TG could be attributed to a simple enhancement of compliance in oral hygiene. In the pilot study, no placebo mouth rinse was used in the control group; this would have contributed to a standardisation of the regime and objectified any possible over-motivation (Hawthorne effect). Øgaard et al. [2006] compared the combined use, during treatment with fixed appliances, of an AmF/SnF_2 toothpaste/mouth rinse with a NaF toothpaste/mouth rinse in orthodontic patients. The use of an AmF/SnF_2 toothpaste/mouth rinse had a slightly stronger decreasing effect on plaque scores in the upper anterior region compared with NaF products. Beneficial effects on gingival health after placement of fixed orthodontic appliances were also observed by Madléna et al. [2012]. Stannous fluoride has a well-known plaque-inhibiting effect and may impede bacterial metabolism [Baehni and Takeuchi, 2003]. Microbial parameters were not collected in the study conducted by Øgaard et al. [2006] and Madléna et al. [2012]. Concerning the effects of fluoride mouth rinse on the salivary MS, Yoshihara et al. [2001] and Kaneko et al. [2006] concluded that a NaF mouth rinse might contribute to a reduction of the number of MS. In the present pilot study, no clear evidence of the NaF mouth rinse on the monitored microbial parameters could be demonstrated. This may be due to the relatively short observation time and the small sample size in comparison to the studies undertaken by Yoshihara et al. [2001] and Kaneko et al. [2006].

Apart from microbial aspects, the effectiveness of a fluoride mouth rinse on caries prevention results mainly from the enhancement of the process of remineralisation [ten Cate, 2001]. In the present pilot study, the number of clinically visible initial caries lesions decreased in five patients (20.8%) within the test group. On the other hand, an increased number of IL was only found in patients within the control group. Additional studies with larger study groups should be conducted using a placebo mouth rinse to evaluate the effect of the fluoride mouth rinse on patients with removable orthodontic appliances.

Conclusions

1. The present longitudinal study suggests that patients wearing removable orthodontic appliances exhibit high levels of salivary mutans streptococci and lactobacilli together with high plaque accumulation.
2. Associations were found between levels of salivary mutans streptococci and lactobacilli and API. Associations could be shown between salivary mutans streptococci and PBI values.
3. The wear-time of removable orthodontic appliances seems to affect the levels of salivary mutans streptococci and lactobacilli and API values, and should be considered a confounding factor in future oral hygiene studies.
4. The efficacy of a fluoride mouth rinse will have to be addressed in future studies with larger sample size and a placebo control.

Finally, the results emphasise the need for a careful monitoring of the oral hygiene status in patients with removable orthodontic appliances. This may also apply to children treated with removable dentures who also wear the respective appliances for extended time spans during the day.

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References

- Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of Candida and plaque in adolescents. Br J Orthod 1982;9:158-163.
- Antoszewska J, Kawala B, Minch L. Selected aspects of the oral environment in cleft palate patients-a problem evidently beyond dentists' scope. Postepy Hig Med Dosw (Online) 2010;64:659-664.
- Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis 2003; 9 Suppl 1:23-9.
- Batoni G, Pardini M, Giannotti A, Ota F, Giuca MR, Gabriele M, Campa M, Senesi S. Effect of removable orthodontic appliances on oral colonisation by mutans streptococci in children. Eur J Oral Sci. 2001;109:388-392.
- Benson PE, Parkin N, Millett DT, Dyer FE, Vine S, Shah A. Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database Syst Rev. 2004: CD003809.
- Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Res. 2005;39:41-47.
- Kaneko N, Yoshihara A, Ida H, Nomura Y, Imai S, Nisizawa T, Sakuma S, Hanada N, Miyazaki H. Influence of a fluoride mouthrinse on mutans streptococci in schoolchildren. Caries Res 2006;40:501-507.
- Karjalainen S, Soderling E, Pienihakkinen K. Validation and inter-examiner agreement of mutans streptococci levels in plaque and saliva of 10-year-old children using simple chair-side tests. Acta Odontol Scand 2004;62:153-157.
- Kneist S, Kubieziel H, Willershausen B, Kupper H, Callaway A. Modeling of S mutans and A naeslundii acid production in vitro with caries incidence of low- and high-risk children. Quintessence Int 2012;43:413-420.
- Kneist S, Schmidt F, Callaway A, Willershausen B, Rupf S, Wicht M, Thiede B. Diversity of Lactobacillus species in deep carious lesions of primary molars. European Archives of Paediatric Dentistry 2010;4:181-186.
- Krasse B. Biological factors as indicators of future caries. Int Dent J 1988;38:219-225.
- Lange DE, Plagmann HC, Eenboom A, Promesberger A. Clinical methods for the objective evaluation of oral hygiene. Dtsch Zahnarztl Z 1977;32:44-47.
- Madléna M, Banoczy J, Gotz G, Marton S, Kaan M Jr, Nagy G. Effects of amine and stannous fluorides on plaque accumulation and gingival health in orthodontic patients treated with fixed appliances: a pilot study. Oral Health Dent Manag 2012;11:57-61.
- McCracken GI, Janssen J, Steen N, DeJager M, Heasman PA. A clinical evaluation of a novel data logger to determine compliance with the use of powered toothbrushes. J Clin Periodontol 2002;29:838-843.
- Øgaard B, Alm AA, Larsson E, Adolfsson U. A prospective, randomized clinical study on the effects of an amine fluoride/stannous fluoride toothpaste/mouthrinse on plaque, gingivitis and initial caries lesion development in orthodontic patients. Eur J Orthod 2006;28:8-12.
- Saxer UP, Mühlemann HR. Motivation and education. SSO Schweiz Monatsschr Zahnheilkd 1975;85:905-919.
- Schlagenhauf U, Tobien P, Engelfried P. Effects of orthodontic treatment on individual caries risk parameters. Dtsch Zahnarztl Z 1989;44:758-760.
- Stösser L, Kneist S, Heinrich-Weltzien R, Fischer T, Tietze W. Current Research on Caries Risk Assessment. In: Stookey, G. K. (ed.): Early Detection of Dental Caries II: Proceedings of the 4th Annual Indiana Conference Indianapolis, India. Indianapolis, IN: Indiana University School of Dentistry, 2000. pp.31-56.
- Tanabe Y, Park JH, Tinanoff N, Turng BF, Lilli H, Minah GE. Comparison of chairside microbiological screening systems and conventional selective media in children with and without visible dental caries. Pediatr Dent 2006;28:363-368.
- ten Cate JM. Consensus statements on fluoride usage and associated research questions. Caries Res 2001;35 Suppl 1:71-73.
- Topaloglu-Ak A, Ertugrul F, Eden E, Ates M, Bulut H. Effect of orthodontic appliances on oral microbiota--6 month follow-up. J Clin Pediatr Dent 2011;35:433-436.
- Yoshihara A, Sakuma S, Kobayashi S, Miyazaki H. Antimicrobial effect of fluoride mouthrinse on mutans streptococci and lactobacilli in saliva. Pediatr Dent 2001;23:113-117.
- Zeger SJ, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988;44:1049-60.