Effect of Orthodontic Appliances on Oral Microbiota—6 Month Follow-up

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***Objective**: This study investigates the effect of fixed and removable orthodontic appliances among children on salivary Streptococcus mutans, Lactobacillus sp. and Candida albicans. **Study Design**: The study was conducted on 69 patients aged between 6-17 years who used fixed or removable orthodontic appliances. Five ml samples of unstimulated saliva from each patient were collected at baseline and at the 1, 3 and 6 month periodic controls. Samples were diluted and plated on Mitis Salivarus Agar (MSA), Man Rogosa Sharp Agar (MRS) and Saboroud Dextrose Agar (SDA). The growths on the plates were examined under a stereomicroscope. Numbers of colony forming units (CFU) per plate were counted. For statistical analysis, the paired t test and Chi-Square were used. **Results**: S mutans and Lactobacillus sp counts increased significantly 6 months after the insertion of fixed/removable orthodontic appliances in the oral cavity. A significant increase for C albicans presence was noted after 3 months compared with baseline for fixed appliances. **Conclusion**: Long-term utilization of orthodontic appliances may have a negative effect on microbial flora and increase the risk of new carious lesions and periodontal problems. Patients should be recalled within short time intervals to be motivated for oral hygiene during their orthodontic therapy.*

***Keywords**: orthodontic appliance, S mutans, Lactobacilli sp, C albicans*
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INTRODUCTION

Ecological equilibrium maintains the healthy oral status of individuals. When this equilibrium is broken, periodontal diseases and dental caries may occur.[1] Modification of the oral microbiota is strongly associated with the application of orthodontic appliances.[2] Insertion of orthodontic appliances into the oral cavity greatly inhibits oral hygiene and increases the number of plaque retention areas.[3] These changes in the oral environment are followed by an increase in bacterial concentration, alterations in the buffer capacity, pH acidity and the salivary flow rate.[4]

The influence of fixed and removable orthodontic appliances on the number of opportunistic bacteria in the oral cavity has been studied and viability of *S mutans* and *Lactobacilli sp.* have been demonstrated.[5] However, the changes observed in bacterial counts between pretreatment and during treatment varies among studies. In contrast to findings reporting an increase in *S mutans* counts during orthodontic therapy,[4,6-9] some investigators reported no change.[10-12] Likewise, there was discord between studies indicating an elevation in *lactobacillus sp* levels[4,8,9,11] and studies revealing no difference after the insertion of orthodontic appliances.[10,12] The inconsistencies between these findings remain unclear and should be studied further.

In recent years, effect of orthodontic appliances on *Candida* carriage status in the oral cavity has been investigated. However, the number of such studies remains limited.[13-17]

The aim of the present study was thus to investigate the effect of fixed and removable appliances among children on *S mutans*, *Lactobacillus sp.* counts and *C albicans* presence in saliva during the first 6 months of the therapy.

SUBJECTS AND METHODS

Study Group

Ethical approval for conducting the study was obtained from the Ethics Committee of Ege University, Izmir, Turkey (reference number: 08-5.1/4). Children who needed orthodontic treatment, who were free of systemic disease and had not used antibiotics and/or steroids within the last 3 months, were included in the study. Participants were asked to inform us if they had used any kind of medication or antibacterial agents such as mouth rinses during the trial procedure. Any

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use of medications was reported and participants were excluded from the study. Signed consent forms were collected prior to the commencement of the study. Sixty-nine participants (31 girls and 38 boys) between the ages of 6-17 years old made up the study group; they were recruited from Ege University School of Dentistry Pedodontics and Orthodontics Department. Subjects were examined intraorally and radiologically with regard to indication of orthodontic treatment and subsequently grouped as patients to be treated with removable (Group I, n=34) or fixed (Group II, n=35) orthodontic appliances. Before the insertion of appliances, all subjects received oral hygiene instructions that included the correct use of a toothbrush. All participants' oral hygiene was considered to be ideal at the beginning of the therapy.

Salivary Sample collection

Five ml of unstimulated whole saliva was collected from each patient at baseline and at the 1, 3 and 6-month recalls. Participants attended between 9:00-10:00 AM and were asked to sit quietly and accumulate saliva for 5 minutes at each evaluation period.

Bacteriological evaluation

For bacterial analyses, samples were processed in the laboratory within 30 minutes and were diluted and plated on MSA (with tellurite and bacitrasin), MRS agar and SDA. MSA and MRS were incubated anaerobically (with 5% CO_2) for 48 hours whereas SDA was incubated for 72 hours at 37°C. The growth on the plates was examined under the stereomicroscope. The number of colony forming units (CFU) per plate was counted and transformed to CFU/sample. The total CFU were obtained from the blood agar plates.

Statistical Analysis

Statistical analysis was carried on bacterial counts, expressed as values of logarithm 10 ($\log_{10}$) colony forming units (CFU). The statistical comparison of pre- and post-treatment bacteria counts in saliva was done using the paired *t*-test. The presence of *C albicans* through all time intervals was analyzed using Chi-Square.

RESULTS

At baseline, among 34 saliva samples from the removable appliance group, one saliva sample could not be bacteriologically evaluated due to the inadequate amount of saliva collected. Consequently, 35 saliva samples from the fixed and 33 from the removable appliance groups were evaluated at baseline. The numbers and proportions of subjects at the 1, 3 and 6 month controls are given in Table 1.

There was no statistically significant difference found between bacteriological counts of subjects with fixed and removable appliances at any evaluation period ($p>0.05$).

In the presence of a fixed/removable orthodontic appliance, no difference was found in *S mutans* and *Lactobacillus sp.* counts between the baseline, 1st and 3rd months' values. However, 6 months after the insertion of the orthodontic appliance in the oral cavity, statistically significant increases in *S mutans* and *Lactobacilli sp.* counts were reported ($p<0.05$) (Table 2, 3).

In the removable appliance group, there were no differences in the presence of *C albicans* over time (Fig. 1). *C albicans* presence was statistically significantly different between fixed and removable appliances at all evaluation periods ($p<0.05$). The number of subjects with *C albicans* present in saliva was higher in the fixed appliance groups compared with the removable appliance groups. At the one-month control, there was no difference in salivary *C albicans* presence, but after 3 months, a statistically significant increase in the number of subjects with salivary *C albicans* was noted compared with baseline. Six months after orthodontic therapy with fixed appliances, no difference in *C albicans* presence was reported (Fig. 2).

Table 1. Number and proportions of subjects at 1, 3 and 6 month control visits.

	Baseline N (%)	1st month N (%)	3rd month N (%)	6th month N (%)
Removable orthodontic appliances	33 (100%)	27 (82%)	20 (60%)	10 (30%)
Fixed orthodontic appliances	35 (100%)	31 (89%)	20 (57%)	8 (22%)

Table 2. Means and standard deviations of *S. mutans* expressed as $\log_{10}$ CFU
$^{a,c}p<0.05$, $^{b,d}p>0.05$

	S. mutans Baseline	*S. mutans* 1st month	*S. mutans* 3rd month	*S. mutans* 6th month
Removable orthodontic appliances	$4.4\pm1.1^{a,b}$	4.0 ± 1.4^{a}	4.4 ± 1.1^{a}	5.2 ± 0.6^{b}
Fixed orthodontic appliances	$4.1\pm1.0^{c,d}$	4.2 ± 1.3^{c}	4.4 ± 1.0^{c}	5.5 ± 1.0^{d}

Table 3. Means and standard deviations of *Lactobacillus sp* expressed as $\log_{10}$ CFU
$^{a,c}p<0.05$, $^{b,d}p>0.05$

	Lactobacillus sp Baseline	*Lactobacillus sp* 1st month	*Lactobacillus sp* 3rd month	*Lactobacillus sp* 6th month
Removable orthodontic appliances	$5.6\pm1.2^{a,b}$	5.4 ± 1.4^{a}	5.8 ± 1.3^{a}	6.6 ± 0.7^{b}
Fixed orthodontic appliances	$5.7\pm1.0^{c,d}$	5.9 ± 1.4^{c}	6.0 ± 1.1^{c}	6.3 ± 0.6^{d}

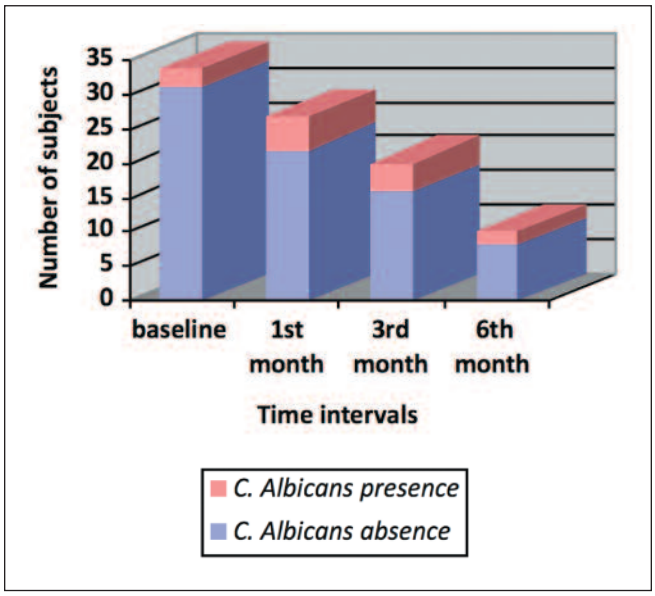

Figure 1. *C albicans* growth through baseline and 1, 3 and 6 month intervals for removable orthodontic appliances

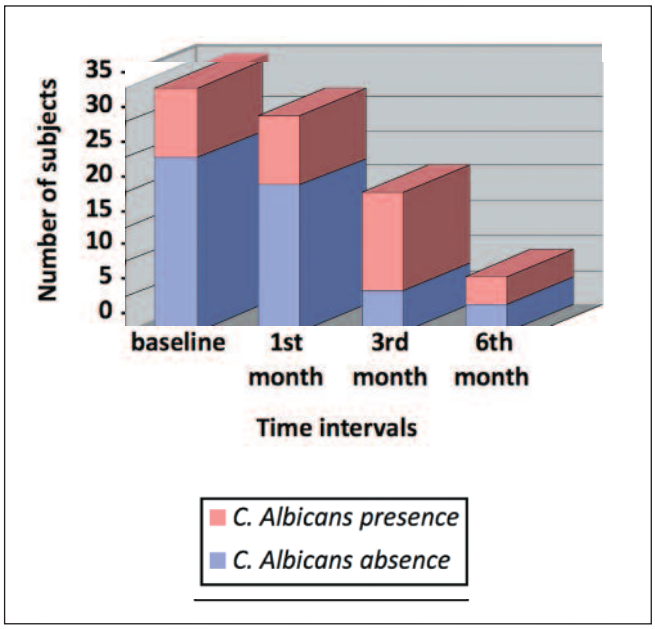

Figure 2. *C albicans* growth through baseline and 1, 3 and 6 month intervals for fixed orthodontic appliances

DISCUSSION

The oral environment has the capacity to adjust to the presence of an orthodontic appliance. This has been shown by studies reporting an increase in stimulated flow rate, buffer capacity and salivary pH that enhance the anticaries properties of saliva.[4,12] These alterations are physiologic responses to maintain the oral health in adverse situations by avoiding potentially pathogenic microorganism colonization, acidogenic bacteria products and demineralization.[12]

However, it is documented that orthodontic appliances may increase the number of sites where plaque can accumulate and alter bacterial flora. This may promote an increase in the risk of carious lesion.[18] Thus, it is assumed that the balance between protective function of the saliva and cariogenic challenge posed by increased bacterial concentration determines the likelihood of demineralization or remineralization.[4]

Our results indicate that, 6 months after insertion of fixed/removable orthodontic appliance, the microscopic counts of oral bacteria increased. It is reported that, patients with fixed orthodontic appliances have an increased risk of white-spot lesion formation due to the changes in the oral microbiota, particularly with respect to *S mutans* which has a key role in caries formation process.[19,20]

The findings of the present study are in line with the studies reporting that orthodontic treatment may affect the oral microbiota equilibrium, as bands, brackets, wires and acrylic resins increase the risk of retention of food particles and microorganisms.[19,22-24]

In the present study, it is also important to underline that at 1 and 3 month controls, there was no difference in *S mutans* and *Lactobacillus sp* counts. However, at the 6 month control, there was a significant increase in both *S mutans* and *Lactobacillus sp*. counts. This could be attributed to the fact that the subjects of the study initially were very well motivated and recall periods were shorter, which might have encouraged the patients to apply oral hygiene instructions routinely so that no changes in the bacteriological counts were observed. A longer period of time might also have helped to bring about a notably higher bacterial colonization at the 6-month control. Our results are in line with studies reporting an elevation in *S mutans* and *lactobacillus sp* counts during therapy[4,6-9] and do not accord with other studies indicating no change.[10-12] This inconsistency may be due to different sampling techniques, different study designs and the numbers of attachments used in appliances.

Studies reveal that high colonization by the fungal pathogen *Candida albicans* is frequently detected in orthodontic patients. Orthodontic appliances were reported to increase the oral *candida* carriage rate and lead to an alteration in *candida* counts during treatment. Our results are in line with other studies documenting an increase in Candida growth.[14,17]

This increase in the number of subjects with salivary *C albicans* present was observed at the 3-month control but was not apparent at the 6-month control. This fluctuating pattern could be a consequence of the high number of dropouts observed at the 6-month control. The high dropout rate was observed among children who did not comply with the protocol for users of both fixed and removable orthodontic appliances. The most significant reason is that, during the 6 months period, many children had used medications like antibiotics for diseases like upper respiratory infection, bad throat, etc. Some patients also used mouthrinses whereas some others did not use their removable appliance regularly. Hence, saliva samples were not obtained from these patients to control the measurement bias. A very small fraction of the dropouts was due to failure to keep appointments.

Recent studies reveal that any foreign objects in the mouth, whether they are fixed or removable appliances, seem to alter the microbiological environment by providing suitable adherence surfaces for *Candida*.[13,14,21] In the present study and compared with removable appliances, fixed orthodontic appliances lead to high numbers of subjects infected with *C albicans*. More participants seemed to change from being non-Candida carriers to Candida carriers status after wearing fixed orthodontic appliance. An increase in the number of patients with candida does not imply that these patients will develop candidosis, but their risk of infection will be increased, particularly if their immune defense has been undermined by factors such as antibiotic usage and local trauma from the appliances.[21] Aspiration of organisms from the oral cavity into the respiratory tract can cause systemic infections such as respiratory problems and heart disease.[2] A more cautious approach for immunocompromised orthodontic patients to avoid candidal infections is warranted.

CONCLUSIONS

Fixed and removable orthodontic appliances have the potential to increase the bacterial and fungal population. Hence, clinicians must preserve the patient's oral health during long orthodontic treatment by monitoring the oral hygiene of the patients during periodic recalls and motivating for oral hygiene to avoid the risk of new caries and periodontal diseases.[17,18] Prevention also implies adequate local fluoride administration, notably the application of fluoride-rich varnish. Other prophylactic measures such as using chlorhexidine varnish and sealing dental grooves are also recommended. Eventually, items likely to retain dental plaque such as bands and elastomeric ties can also be minimized by better design of orthodontic appliances.[18,25,26]

REFERENCES

1. Petti S, Renzini G. Comparison between two different methods to evaluate the rate of anaerobic microorganisms in the subgingival microflora. Minerva Stomatologica, 43: 89–94, 1994.
2. Kitada K, de Toledo A, Oho T. Increase in detectable opportunistic bacteria in the oral cavity of orthodontic patients. Int J Dent Hygiene, 7: 121–125, 2009.
3. Bollen AM, Cunha-Cruz J, Bakko DW, Huang GJ, Hujoel PP. The effects of orthodontic therapy on periodontal health. A systematic review of controlled evidence. J Am Dent Assoc, 139: 413–422, 2008.
4. Chang HS, Walsh LJ, Freer TJ. The effect of orthodontic treatment salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. Aust Orthod, 15: 229–234, 1999.
5. Kupietzky A, Majumdar AK, Shey Z, Binder R, Matheson PB. Colony forming unit levels of salivary Lactobacilli and Streptococcus mutans in orthodontic patients. J Clin Pediatr Dent, 30: 51–53, 2005.
6. Rosenbloom RG, Tinanoff N. Salivary Streprococcus mutans levels in batients before, during, and after orthodontic treatment. Am J Orthod Dentofacial Orthop, 100: 35–35, 1991.
7. Scheie AA, Arneberg P, Krogstad O. Effect of orthodontic treatment on prevalence of Streptococcus mutans in plaque and saliva. Scand J Dent Res, 92: 211–217, 1984.
8. Sari E, Birinci I. Microbiological evaluation of 0.2% chlorhexidine gluconate mouth rinse in orthodontic patients. Angle Orthod, 77: 881–884, 2007.
9. Cheng LL, Moor Sl, Kravchuk O, Meyers IA, Ho CT. Bacteria and salivary profile of adolescents with and without cleft lip and/or palate undergoing orthodontic treatment. Aust Dent J, 52: 315–321, 2007.
10. Ulukapi H, Koray F, Efes B. Monitoring the caries risk of orthodontic patients. Quintessence Int, 28: 27–29, 1997.
11. Sanpei S, Endo T, Shimooka S. Caries risk factors in children under treatment with sectional brackets. Angle Orthod, 80: 509–514, 2010.
12. Lara–Carillo E, Montiel-Bastida NM, Sanchez-Perez L, Alanis-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of Streptococcus mutans and Lactobacillus. Med Oral Pathol Oral Cir Bucal-ahead of print.
13. Arendorf TM, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol, 12: 360–368, 1985.
14. Hagg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. Eur J Orthod, 26: 623–629, 2004.
15. Lee W, Low BKM, Samaranayake LP, Hagg U. Genotypic variation of Candida albicans during orthodontic therapy. Front Biosci, 13: 3814–3824, 2008.
16. Brusca MI, Chara O, Sterin-Borda L, Rosa AC. Influence of different orthodontic brackets on adherence of microorganisms in vitro. Angle Orthod, 77: 331–336, 2007.
17. Arslan SG, Akpolat N, Kama JD, Özer T, Hamamcı O: One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral *candida.* J Oral Pathol Med. 37: 26–29, 2008.
18. Opsahl Vital S, Haignere-Rubinstein C, Lasfargues JJ, Chaussain C: Caries risk and orthodontic treatment. Int Orthod, 8: 28–45, 2010.
19. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod, 81*:* 93–98, 1982.
20. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, Reynolds EC, Morgan MV. Regression of Post-orthodontic Lesions by a Remineralizing Cream. J Dent Res, 88: 1148–1153, 2009.
21. Hibino K, Wong RW, Hägg U, Samaranayake LP. The effects of orthodontic appliances on *Candida* in the human mouth. Int J Paediatr Dent, 19: 301–308, 2009.
22. Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. American Journal of Orthodontics and Dentofacial Orthopedics, 130: 275.e17–e22, 2006.
23. Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. American Journal of Orthodontics, 94: 68–73, 1988.
24. Fournier A, Payant L, Bouclin R. Adherence of *Streptococcus mutans* to orthodontic brackets. American Journal of Orthodontics and Dentofacial Orthopedics, 114: 414–417, 1998.
24. Tronchin G, Bouchara JP, Robert R, Senet JM. Adherence of *Candida albicans* germs tubes to plastic: ultrastructural and molecular studies of fibrillar adhesins. Infect Immun, 56: 1987–1993, 1988.
25. Derks A, Kuijpers Jagtman AM, Frencken JE, Van't Hof MA, Katsaros C. Caries preventive measures used in orthodontic practices: an evidence-based decision? Am J Orthod Dentofacial Orthop, 132: 165–170, 2007.
26. Derks A, Frencken J, Bronkhorst E, Kuijpers Jagtman AM, Katsaros C: Effect of chlorhexidine varnish application on mutans streptococci counts in orthodontic patients. Am J Orthod Dentofacial Orthop, 133: 435–439, 2008.