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Increase in detectable opportunistic bacteria in the oral cavity of orthodontic patients

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Abstract: *Objectives:* This study was performed to detect the opportunistic bacteria and fungi from the oral cavities of orthodontic patients and examine the ability of the organisms to adhere to saliva-coated metallic brackets. *Methods:* Opportunistic bacteria and fungi were isolated from 58 patients (orthodontic group: 42; non-orthodontic group: 16) using culture methods and were identified based on their biochemical and enzymatic profiles. Seven opportunistic and four streptococcal strains were tested for their ability to adhere to saliva-coated metallic brackets. *Results:* More opportunistic bacteria and fungi were detected in the orthodontic group than in the non-orthodontic group ($P < 0.05$). Opportunistic bacteria adhered to saliva-coated metallic brackets to the same degree as oral streptococci. *Conclusions:* The isolation frequencies of opportunistic bacteria and fungi increase during orthodontic treatment, suggesting the importance of paying special attention to oral hygiene in orthodontic patients to prevent periodontal disease and the aggravation of systemic disease in immunocompromised conditions.

Key words: adherence; opportunistic bacteria; oral hygiene; orthodontic patient

Introduction

Opportunistic bacteria and fungi, such as *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Candida albicans* sometimes cause respiratory infections and heart disease in immunocompromised patients (1–8). These bacteria and fungi are generally or

occasionally indigenous in the oral cavity and are detected at higher frequencies when immune response of the patients is reduced. Aspiration of these organisms from the oral cavity into the respiratory tract can cause systemic infections in immunocompromised patients (2, 9). In addition, these organisms have been found to be correlated with periodontal disease (3, 10, 11).

Various studies have examined the effects of orthodontic appliances on the microbial profile of dental plaque. Anhoury *et al.* (12) compared the total number of cariogenic and periodontopathic bacteria on metallic and ceramic orthodontic brackets and found no significant differences between the two types of bracket with respect to the count of cariogenic bacteria. However, Diamanti-Kipioti *et al.* (13) found a significant increase in some species of periodontopathic bacteria after the placement of orthodontic bands in children and Naranjo *et al.* (14) also found that bracket placement influences the accumulation of plaque and the colonization of major periodontopathic bacteria.

When we examined the influence of orthodontic treatment on the numbers of opportunistic bacteria and fungi in the oral cavity, we found more of these organisms in orthodontic patients. Additionally, we discuss the mechanism in relation to the adhering abilities of the organisms to saliva-coated metallic brackets (S-Br).

Material and Methods

Subjects

Fifty-eight patients visiting the Preventive Dentistry Clinic at Kagoshima University Medical and Dental Hospital, Kagoshima, Japan participated in this study (Table 1). They regularly received oral hygiene care such as tooth brushing instructions, scaling and professional teeth cleaning. Forty-two patients also underwent orthodontic treatment in the Orthodontic Dentistry Clinic. The orthodontic group was divided into two subgroups: one wore multibracket appliances (MB group) and the other wore other appliances such as Hawley's type retainer, Begg's type retainer, fixed type retainer, activator, quad helix appliance, lingual arch appliance and maxillary protracting appliance. All of the subjects in the MB group were treated with metallic brackets. There was no difference in treatment procedure among three groups. All the patients received dental caries preventive program including topical fluoride application. The Ethics Committee of Kagoshima University Medical and Dental Hospital, Kagoshima, Japan, approved the experimental protocol (reference number 19–95) and informed consent was obtained from all subjects prior to their participation in this study.

Table 1. **Plaque control record of patients with and without orthodontic treatment**

	Orthodontic		
	MB	Others	Non-orthodontic
Subjects (*n*)	24	18	16
Age (mean ± SD)	20.0 ± 6.9	17.2 ± 11.1	33.8 ± 19.1
Plaque control record (%, mean ± SD)	44.5 ± 15.8	37.2 ± 17.0	25.3 ± 19.8

MB, multi-bracket appliances; Others, other appliances.

Detecting opportunistic bacteria and fungi in the oral cavity

On a visit to the Preventive Dentistry Clinic, the subjects were first examined for dental plaque deposition using the plaque control record proposed by O'leary *et al.* (15). Next, the subjects underwent professional tooth brushing on the buccal, lingual and occlusal surfaces and tongue dorsum, and flossing in the interproximal tooth surfaces for 1 min by a trained dentist. Subsequently, they rinsed the oral cavity with 5 ml of sterilized saline (16) and expectorated into a tube. The expectorated fluid was absorbed using a cotton swab, transferred to a transport medium (Seedswab γ No. 3; Eiken Chemical Co., Ltd., Tokyo, Japan) and kept at 4°C. The samples were immediately transported to the Clinical Pathology Laboratory Inc. (Kagoshima, Japan), where the isolation frequencies of bacteria and fungi in each were determined. Briefly, the samples were plated directly onto chocolate agar and blood agar plates and were incubated in an atmosphere of 5% CO_2 at 37°C for 48 h. Colonies on these plates were differentiated by their macroscopic appearance (form, haemolysis), Gram stainability, catalytic reaction and oxidase reactions (17–20). Following the examination, an isolate of each colony type was identified using the VITEK® system (BioMérieux, Marcy l'Etoile, France) to generate biochemical and enzymatic profiles.

Bacterial adherence to S-Br

Eleven strains were used. *E. cloacae* IID977 (ATCC13047), *K. pneumoniae* IID5209 (ATCC15380), *P. aeruginosa* IID1030 (ATCC27107), *Ser. marcescens* IID5218 (ATCC274) and *Strep. pneumoniae* GTC261 (NCTC7465) were purchased from Japanese Society for Bacteriology (Tokyo, Japan). *Staphylococcus aureus* ATCC21027, *Staph. epidermidis* ATCC155, *Streptococcus anginosus* ATCC33397, *Streptococcus salivarius* ATCC13149, *Streptococcus sanguis* ATCC10556 and *Streptococcus mutans* MT8148 were obtained from the stock culture collection at

the Department of Preventive Dentistry, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

Paraffin-stimulated whole saliva was collected from a healthy donor (a 25-year-old female) in an ice-chilled tube and clarified by centrifugation at 12 000 *g* for 20 min at 4°C. In the preliminary experiment, we observed no particular difference in the ability to induce bacterial adherence among several saliva samples. Therefore, we used single donor sample as a representative.

The adherence assay was performed using a previously described method (21). Briefly, a metallic bracket (MINI UNI-TWIN® for upper left central incisor; 3M Unitek Orthodontic Products, Monrovia, CA, USA) was incubated with 200 μl of clarified whole saliva for 1 h at 37°C and washed three times with buffered KCl (22). The bacterial cells were labelled with 2′,7′-bis[2-carboxyethyl]-5[6]-carboxyfluorescein (BCECF). Bacterial cells were grown in Todd-Hewitt broth (Becton Dickinson, Sparks, MD, USA) at 37°C for 18 h anaerobically and BCECF acetoxymethyl ester (Sigma Chemical, St. Louis, MO, USA) was added to the bacterial culture to a final concentration of 10 μmol l^{-1} and incubated for an additional 30 min in the dark. After incubation, the cells were harvested by centrifugation and were washed three times with buffered KCl. BCECF-labelled bacteria (4×10^8 cells) were allowed to react with S-Br in 200 μl buffered KCl at 37°C for 3 h. After incubation, the bracket was rinsed three times with buffered KCl and the fluorescence intensity associated with the S-Br was determined using a multi-label counter (Wallac 1420 ARVO®; Wallac, Turku, Finland). The number of bacteria bound was determined using a standard plot of the number of bacterial cells against the fluorescence intensity.

Statistical analysis

The data for the mean age and plaque control record of the subjects were analysed using ANOVA and Bonferroni's multiple *t*-test. The data for the age distribution and isolation frequencies of opportunistic bacteria and fungi were compared using the chi-squared test (SPSS 11.0J for Windows; SPSS Japan Inc., Tokyo, Japan).

Results

Detection of opportunistic bacteria and fungi

There were no significant differences in the mean age and age distribution among three groups (Table 1). To evaluate oral hygiene, plaque control record was examined for each patient. No statistically significant differences were observed in the record among three groups.

Table 2. **Isolation frequencies of opportunistic bacteria and fungi**

	Number of subjects		
	Orthodontic		
Bacteria and fungi	MB	Others	Non-orthodontic
Enterobacter cloacae	6	2	0
Klebsiella pneumoniae	1	1	0
Pseudomonas aeruginosa	2	0	0
Serratia marcescens	0	1	0
Staphylococcus aureus (MSSA)	0	1	1
Staph. epidermidis	0	2†	0
Streptococcus pneumoniae	0	0	0
Candida albicans	1	0	0
Total*	10	6	1

MB, multi-bracket appliances; Others, other appliances; MSSA, methicillin-suspectible *Staphylococcus aureus*.
*$P < 0.05$ between orthodontic group and non-orthodontic group (chi-squared test).
†One is the same subject who possesses *Serratia marcescens* isolate.

The isolation frequencies of opportunistic bacteria and fungi in the oral cavity are shown in Table 2. These organisms were detected from 16 subjects in the orthodontic group and one subject in the non-orthodontic group. In the MB group, *E. cloacae* were detected most frequently in six subjects and *P. aeruginosa* was detected in two subjects. In the other appliances wearing group, *E. cloacae* and *Staph. epidermidis* were detected in two subjects each. In contrast, only one bacterium (*Staph. aureus*) was detected from one subject in the non-orthodontic group. We detected a statistically significant difference in the isolation frequencies between the orthodontic and non-orthodontic groups ($P < 0.05$).

Adherence of the bacteria to S-Br

The adherence of opportunistic bacteria and several oral streptococci to S-Br are shown in Figure 1. Of the opportunistic bacteria, a strain of *P. aeruginosa* adhered most strongly to S-Br and strains of *K. pneumoniae* and *E. cloacae* also adhered strongly. The adhering abilities of these three strains were the same or greater than for the oral streptococcal strains tested. Conversely, the strains of *Strep. pneumoniae* and *Staph. aureus* adhered to S-Br weakly.

Discussion

In this study, we found that the isolation frequencies of opportunistic bacteria and fungi in the oral cavity of the orthodontic

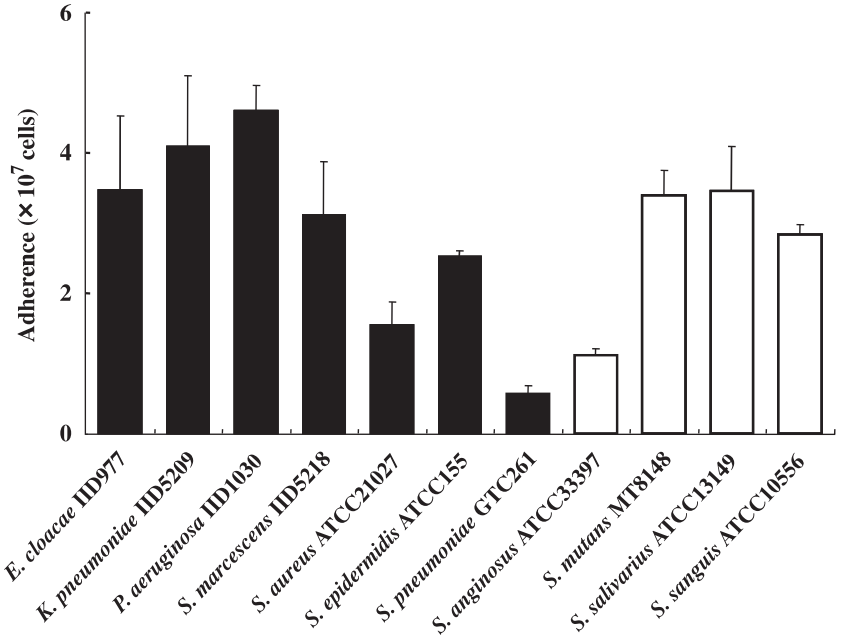

Fig. 1. Adherence of opportunistic bacteria and oral streptococci to saliva-coated metallic brackets. The values are the means ± SD of triplicate assays.

group were higher than those in the non-orthodontic group. Opportunistic bacteria and fungi are frequently detected in immunocompromised patients and the elderly, and orthodontic treatment using appliances might induce a critical oral environment in which opportunistic organisms can grow and survive. Of course, the placement of orthodontic appliances makes it difficult to remove dental plaque using a toothbrush and dental floss. The orthodontic patients in this study received professional teeth cleaning every 1 or 2 months. Although no significant differences were observed in the plaque control record among the three groups (Table 1), the isolation frequencies of the organisms differed significantly between the orthodontic and non-orthodontic groups (Table 2).

In the MB group, several opportunistic bacteria were isolated. To elucidate the mechanism of this phenomenon, we examined the adhering abilities of organisms to S-Br *in vitro*. Strains of *E. cloacae*, *K. pneumoniae* and *P. aeruginosa* adhered to S-Br strongly, and these three species were detected from the MB group *in vivo*. Conversely, strains of *Strep. pneumoniae* and *Staph. aureus* adhered to S-Br weakly *in vitro*, and these organisms were not isolated from the MB group. These results suggest that *Enterobacter*, *Klebsiella* and *Pseudomonas* species have a greater capacity of adhering to S-Br than other opportunistic bacteria. *Streptococcal* species are the major component of dental plaque, and some species are thought to be early colonizers on tooth surfaces coated with salivary proteins (23, 24). Several studies have also shown that cariogenic bacteria such as *Strep. mutans* adhered to orthodontic brackets (25–27). Compared with the oral streptococcal strains used in this study, the opportunistic strains showed almost equal adhering abilities to S-Br. The high detection frequency of opportunistic bacteria from the MB group seems to be related to the adhering abilities of the organisms to metallic brackets.

Opportunistic bacteria have been implicated as causative pathogens that induce health care-associated infections in critically ill or immunocompromised patients (28). These organisms also cause device-associated nosocomial infections, and attempts have been made to develop methods for disease control (29, 30). If the orthodontic appliance-wearers are healthy in general condition, they are treated in the same manner as that of non-orthodontic patients. However, when the wearers become seriously immunocompromised, special attention should be paid to prevent further illness that might be caused by the increase in opportunistic bacteria and fungi. Hence, we strongly recommend that the orthodontic appliances should be removed in such cases.

Generally, adult periodontitis is supposed to be caused by periodontopathic bacteria, such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythensis*, *Fusobacterium nucleatum* and *Treponema denticola*. In addition, studies have reported that opportunistic bacteria are related to periodontal disease. Slots *et al.* (10, 11) suggested that yeasts, enteric rods (i.e. members of the family Enterobacteriae) and pseudomonads, which are opportunistic pathogens, occur in the subgingival flora of about one-third of 'refractory' adult periodontitis patients. Enterobacteriae and pseudomonads, especially *E. cloacae*, *K. pneumoniae* and

P. aeruginosa were detected frequently. Regarding orthodontic treatment, Naranjo *et al.* (14) reported that bracket placement influences the plaque accumulation and colonization of periodontopathic and superinfecting bacteria, resulting in more inflammation of gingival tissue. Based on the latter three reports, we examined opportunistic or superinfecting pathogens, and we think it is necessary to reduce plaque accumulation during orthodontic treatment to prevent such type of periodontal disease.

In conclusion, the isolation frequencies of opportunistic bacteria and fungi increased during orthodontic treatment, and their ability to adhere to S-Br seemed to reflect this phenomenon. Special physical and chemical hygiene care in the oral cavity should be considered for orthodontic patients to prevent periodontal disease and the aggravation of systemic diseases in immunocompromised conditions.

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