Investigation of bacteremia after toothbrushing in orthodontic patients

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Objective: The aim of this study was to investigate the occurrence rate of bacteremia following toothbrushing with toothpastes composed of several antibacterial agents and compare the results with the conventional oral hygiene maintaining methods in orthodontic patients. **Methods:** This clinical study included 100 adult orthodontic patients who were divided into 4 groups. Each group comprised of 25 patients, wearing fixed orthodontic appliances. In the first group, bacteremia was assessed after toothbrushing without using any toothpaste. In the second group, a 0.2% chlorhexidine gluconate mouthrinse was used before brushing with no toothpaste. In the third group, subjects brushed with a commonly used toothpaste which did not include an additional antimicrobial agent. The fourth experimental group used toothpaste which included tea tree oil, clove oil, peppermint oil and bisabolol as antimicrobial elements. Pre- and post-brushing blood samples were obtained using a strict aseptic technique. All samples were microbiologically evaluated using blood culture bottles. **Results:** Toothbrushing in orthodontic patients yielded to an increase in the occurrence rate of bacteremia when using normal toothpaste or no toothpaste at all. **Conclusions:** The use of chlorhexidine mouthwash before toothbrushing, and brushing with antimicrobial toothpaste did not show a statistically significant difference in preventing the occurrence of bacteremia ($p > 0.05$). **(Korean J Orthod 2009;39(3):177-184)**

Key words: Bacteremia, Toothbrushing, Chlorhexidine gluconate, Antibacterial agents

INTRODUCTION

Dental plaque and its composing bacteria were demonstrated to increase after placement of orthodontic appliances, due to the fact that these appliances form retentive areas that cannot be efficiently cleaned by toothbrushing. Bloom and Brown[1] showed that the total oral microflora was greater after orthodontic bands were placed on the teeth than before band placement. They explained this finding by the increase of areas for plaque retention and suggested that the increased number of the organisms was directly related to the number of bands placed in the mouth.

Bacteremia, defined as the presence of viable bacteria in the circulating blood; can result in bloodstream infection, which is one of the most frequent and chal-

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lenging hospital-acquired infections. The microorganisms in the oral cavity can enter the blood stream during dental treatment, oral hygiene procedures or chewing activity, and create a transient bacteremia.[2] It even can occur in healthy populations with manipulation of the oral mucosa, including toothbrushing.[3] Lucas et al.[4] compared baseline values of bacteremia with different types of toothbrushing techniques such as (1) Oral B 30 toothbrush, (2) Braun, (3) Sonicare electric toothbrush or (4) dental handpiece and rubber cup. They found significantly greater aerobic and anaerobic intensity of bacteremia following brushing with both the Sonicare (p = 0.03 and p = 0.05) and the dental handpiece (p = 0.001 and p = 0.005). On the other hand in the pilot study of Misra et al.,[5] the highest incidence of bacteremia (8/11 subjects) was found following brushing with powered toothbrushes, which was significantly ($p < 0.025$) greater than that following brushing with ultrasonic (5/11) or manual (5/11) toothbrushes.

In healthy individuals, the episodes of transient bacteremia last about 10 - 30 minutes, with no dramatic consequences.[6] However, in persons with predisposing factors such as, prosthetic cardiac valves, some forms of congenital heart disease, previous infective endocarditis, and cardiac valvulopathy following cardiac transplantation, transient bacteremia can represent a risk for infective endocarditis. When undertaking dental treatment in these patients, current guidelines of the American Heart Association recommend endocarditis prophylaxis for all dental procedures that involve manipulation of gingival tissue or the periapical region of teeth or perforation of the oral mucosa.[7]

In different studies, the prevalence of bacteremia during orthodontic procedures such as separator placement, band placement, band removal or appliance adjustment has been reported to vary between 0% and 44%.[8-11] Chung et al.[12] studied bacteremia experienced by patients undergoing orthodontic treatment with fixed appliances after toothbrushing. They detected bacteremia in 3 out of 16 patients following toothbrushing. However, it should be noted that they also detected bacteremia in 10 patients before toothbrushing. Schlein et al.[13] studied bacteremia occurring after toothbrushing in twenty patients undergoing orthodontic treatment with fixed appliances and reported that 25% of the sample had positive blood tests after brushing. Lucas et al.[14] studied bacteremia following debanding and gold chain application. They found no significant difference ($p > 0.05$) in the prevalence of bacteremia between baseline (19 per cent) and following upper deband (26 per cent) or between baseline (57 per cent) and gold chain adjustment (57 per cent). They also did not find any significant difference ($p > 0.05$) in the intensity of the anaerobic bacteremia between baseline and following deband or gold chain adjustment. Rosa et al.[15] also claimed a strong possibility that trauma after removing Haas expanders might be correlated to transitory bacteremia. Gürel et al.[16] also claimed similar results as Rosa et al. According to Lucas et al.[17], there was no significant difference in the number of positive blood cultures between baseline (23%), and following an upper alginate impression (31%); between baseline (27%), and placement of a separator (36 per cent); between baseline (36%), and fitting or placement of a band (44%); or between baseline (33%), and archwire adjustment (19.4%). For the separator group only the mean total number of aerobic and anaerobic bacteria combined, isolated from the blood samples (cfu of bacteria per ml of blood), was significantly greater following the placement of a separator (2.2, SD 9.1), compared with baseline (0.9, SD 0.2; $p < 0.02$). As a result of their investigation, it was found that the only orthodontic treatment procedure that causes a significant bacteremia was the placement of a separator. On the other hand Burden et al.[18] found the prevalence of bacteremia detected following debanding considerably lower than reported for dental procedures traditionally covered by antibiotic prophylaxis guidelines.

Antiseptic mouthwashes applied prior to dental procedures were reported to reduce the incidence and severity of bacteremia.[19,20] Erverdi et al.[21] investigated the occurrence rate of bacteremia after orthodontic banding and debanding following a 0.2% chlorhexidine gluconate mouthwash application. They concluded that the application of chlorhexidine mouthwash resulted in a decrease in the prevalence of bacteremia after banding and debanding, which was not statistically significant.

There has been a growing interest in improving

toothpastes by the addition of antimicrobial agents to inhibit plaque, gingivitis and dental caries. Stannous fluoride, oxygenating agents and triclosan used in conjunction with a polymer delivery system, have all been found to be effective in reducing plaque, gingival inflammation and caries in long term studies.[22] In the search for daily use oral care products with minimal side effects, toothpastes and mouthwashes with natural ingredients have been proposed as well. The antimicrobial effect of essential oils such as mastic gum oil, thymol, lavandula oil, tea tree oil and eucalyptus oil against oral pathogens has been demonstrated in various studies.[23-26] The essential oil of Australian tea tree -Melaleuca alternifolia- was shown to have a strong antibacterial activity against periodontopathic and cariogenic bacteria.[27]

The aim of the present study was to find out if there was a difference in the occurrence rate of bacteremia following tooth brushing in orthodontic patients after using, 1) a 0.2% chlorhexidine gluconate mouthwash, 2) a toothpaste without any additional antimicrobial agent 3) an antimicrobial toothpaste which included tea tree oil, peppermint oil, clove oil and bisabolol as its active ingredients.

MATERIAL AND METHODS

A hundred healthy young adult orthodontic patients (45 females and 55 males) whose ages ranged between 17 and 31 comprised the study group. These 100 individuals were selected on the basis that met the inclusion criteria and gave consent for drawing blood.

Patient selection was carried out according to the following criteria: The patients had to be young adults with no history of rheumatic fever, congenital or acquired heart disease, prosthetic valves, bleeding disorders or some other medically compromised situation. Patients who had used antibiotics within the past 3 months and those who were using an antiseptic mouthwash regularly were rejected. Only those individuals with a mean plaque index score of < 2 and a mean modified gingival index score of < 2 were included in the study group.[28,29]

All procedures were explained to the patients and each individual signed an informed consent form in fulfillment of the requirements of the Ethical Committee of Marmara University Institute of Health Sciences. They were instructed not to eat or brush their teeth for 2 hours before their appointments for the drawing blood, and every patient was given a new toothbrush.

This study was carried out in 4 groups, each comprising of randomly allocated 25 patients treated by fixed orthodontic appliances. Pre-angulated and pre-torqued, 0.018 inch metal Edgewise appliances 'Roth System' (Forestadent, Pforzeim, Germany) were used in all patients and they were bonded by regular bracket adhesive including no antibacterial agent on both the upper and lower teeth. The first group was considered as the control group, toothbrushing for 3 minutes without any toothpaste. In the second group, patients were asked to rinse his or her mouth with 15 mL 0.2% chlorhexidine gluconate mouthwash (Klorhex®, Drogsan, Ankara, Turkey) for 60 seconds, before brushing with no toothpaste. Chlorhexidine has been accepted as the golden standard in clinical research studies.[30,31] That is why we also compared an antibacterial toothpaste by switching with chlorhexidine which has already been accepted as a golden standard antibacterial agent. In the third group, subjects brushed for 3 minutes, with a commonly used toothpaste (Ipana® Procter & Gamble, Istanbul, Turkey) which possessed no antibacterial activity other than that provided by 0.32% sodium fluoride only, while the fourth group patients brushed with an antimicrobial toothpaste (One Drop Only®, One Drop Only Gmbh, Berlin, Germany), which included tea tree oil (0.37%), peppermint oil (2.6%), clove oil (concentration not disclosed by the manufacturer) and bisabolol (0.35%).

From each subject, a blood sample of 11 mL was obtained from an antecubital vein before toothbrushing, using a strict aseptic technique. Another 11 mL blood sample was taken within 2 minutes after toothbrushing using the same technique.

Pre- and post brushing blood samples were microbiologically evaluated. Ten milliliters of pre- and post-brushing blood samples were aseptically inoculated into blood culture bottles (Signal Blood Culture System, Oxoid Unipath Limited, Hampshire, England) that were connected to a growth indicator device and incubated at 37°C for 14 days. Positive results were indicated in

the bottles in which the blood and broth mixture had risen above the green locking sleeve of the growth indicator device. Cultures were taken from the positive bottles and plated on agar and blood agar supplemented with 0.0005% hemin (Sigma Chemicals Co, St Louis, Mo) and 0.00005% menadione (Sigma). These were incubated under aerobic and anaerobic conditions, respectively. Bacterial species were identified with colony morphology, gram staining procedures, standard microbiologic biochemical testing technique, and API 20 strips (bioMerieux, Marcy l'Etoile, France). The remaining 1 mL of the pre- and post-brushing blood samples was used to determine the number of bacteria per mL of blood using the pour-plate method which requires 20 mL of fastidious anaerobic agar (Oxoid) supplemented with 5% calf serum.

Odds ratio (OR = ad/bc) was used to see the effects of toothbrushing with regular toothpaste, switching with chlorhexidine after brushing and toothbrushing with antibacterial toothpaste in comparison with the results of the control group (toothbrushing with brush and water only). Chi square test was used for the statistical evaluation of the occurrence rate of bacteremia in the four groups.

RESULTS

The microbiologic findings of this study can be seen in Tables 1 and 2. The control group, where neither toothpaste nor mouthwash was used, showed no pre-brushing bacteremia, while post-brushing bacteremia was evident in the blood samples of 4 patients (16%) of this group. The microorganism isolated from the post-brushing blood sample of the first patient of the control group was *Streptococcus salivarius* while the microorganisms isolated from the second patient were *Streptococcus salivarius* and *Streptococcus mitis*. The microorganisms isolated from the third and fourth patients were *Actinomyces sp*. and *Streptoccocus sanguinis*, respectively.

In the second group where chlorhexidine gluconate was used before toothbrushing, no bacteremia was detected in pre-brushing blood samples, while post-brushing bacteremia was detected in 1 patient (4%) of this group. The microorganism isolated from the post-brushing blood sample of the patient was *Streptococcus salivarius*.

In the Ipana toothpaste group, pre-brushing culture bottles showed no growth, while post-brushing bottles showed bacteremia in 2 patients (8%). The microorganism found was *Streptococcus salivarius* in both

Table 1. Positive blood culture results obtained from the four groups

Group	Control (n = 25)	Chlorhexidine (n = 25)	Ipana (n = 25)	One drop only (n = 25)
Pre-brushing	-	-	-	-
Post-brushing	4 (16%)	1 (4%)	2 (8%)	0 (0%)

Table 2. Microbiologic analysis

Group	Control	Chlorhexidine	Ipana	One drop only
Pre-brushing	-	-	-	-
Post-brushing	*S. salivarius* *S.mitis* *Actinomyces sp.* *S. sanguinis*	*S. sanguis*	*S. salivarius*	-

patients.

No bacteremia could be detected in the pre- and post-brushing cultures of the subjects in the One Drop Only group.

The odds ratio of the chlorhexidine group (OR_1) was 1.14, Ipana group OR_2 was 1.095 and One Drop Only group OR_3 was 1.19. Values greater than 1 show that the experimental group is better than the control group. Since $OR_3 > OR_1 > OR_2$, toothbrushing with an antibacterial toothpaste (One Drop Only) seems to be the most effective oral hygiene procedure to prevent bacteremia.

Although it was possible to distinguish the types of microorganisms that grew in the blood cultures of the four patients in the control and Ipana toothpaste groups, the number of microorganisms was so small that no colony or growth could be counted in terms of colony forming units.

Although we have differences in the results with respect to post-brushing bacteremia: positive in the control group, Ipana toothpaste group, and chlorhexidine gluconate group and negative in the One Drop Only group, the difference between the four groups was not statistically significant ($p > 0.05$).

DISCUSSION

The dental and medical literature provides substantial information on the prophylactic methods to be followed when performing various dental procedures in individuals who are at risk of infective endocarditis. In contrast to the great emphasis given on infective endocarditis originating from dental care, research data showed that only a small percentage of all infective endocarditis cases were related to dental treatment.[6] Some reports have addressed that toothbrushing itself can cause bacteremia[2] and some others not.[3] There is not much information on how to deal with low grade but frequent bacteremia related to everyday events like toothbrushing[12,13,32] or flossing,[33] which have almost the same occurrence rate as dental treatment induced bacteremia and for which antibiotic prophylaxis can not be prescribed. Since bacteremia of this nature is unavoidable, at risk patients and their dental care providers should focus on ways of reducing the number of oral bacteria entering the bloodstream during these activities. There is an apparent need for studies to find out if the use of topical antiseptics during oral hygiene procedures helps to reach this goal or not.

Although most subjects undergoing orthodontic treatment are children and adolescents, this study was preferably conducted on adults. The authors' experience in similar previous studies has shown that parents of underage patients were generally very reluctant to permit their children to participate in a study which required two consecutive blood withdrawal episodes. This reluctancy in participation, which was evident in most adult patients as well, and the difficulty in finding enough subjects fitting the inclusion criteria were limiting factors which precluded the formation of a larger sample size. Power analysis based on this and previous studies show that about 50 subjects would be needed in each group to draw more accurate and reliable conclusions. Given the relatively small sample size, the present study can only be regarded as a pilot study. Collaboration with other institutions can help with increasing the sample size in future studies. Moreover antibacterial agents can decrease the amount of bacteria in saliva or dental plaque. This study might have provided more valuable information if the amount of bacteria in saliva or dental plaque as well as in blood had been measured and compared.

The factors that influence the success of studies which aim to isolate bacteria from the bloodstream were reported to be: 1) site of blood withdrawal, 2) timing of blood withdrawal, 3) blood volume sample, and 4) culturing medium.[13] All of these factors were carefully evaluated when designing the present study. Blood was collected from the antecubital fossa, which was reported to yield colony counts only slightly lower than arterial blood.[34] Blood sampling was performed within two minutes after toothbrushing. It was reported that blood sampling had to be carried out as quickly as possible after the operation, as the chances for detection of bacteremia decreased with each passing minute.[35-37] Although thirty seconds post-procedure is the time used in most recent investigations[18,38] blood sampling in such a short time was not feasible in the present study. The time interval between completion of brushing and initiation of postoperative blood with-

drawal was usually longer than 30 seconds since finding the vein could take some time in a number of patients. This slight delay could have been eliminated by using a cannula during blood sampling; however, cannula insertion requires greater dexterity on the operator's part and it might be perceived by most patients as a more invasive procedure than venipuncture by syringe needle. Increasing the volume of blood sample was shown to provide higher yields of microorganisms.[39] The volume of blood sampled from each patient was sufficient for the purposes of the present study.

Blood culture system used in the present study is a contemporary and sensitive test for detection of bacteremia. On the other hand, the validity of the pour plate method which has been used to determine the intensity of bacteremia can be debated. In recent studies, the technique of lysis filtration has been reported to be a more accurate method for detecting the intensity of bacteremia compared to other methods.[17,38]

The organisms isolated in the present study, *S. salivarius* and *S. mitis*, are viridans streptococci prominent in the oral cavity. *S. sanguinis* and *S. mitis* are the streptococcal species most frequently isolated from patients with infective endocarditis.[40] These species of streptococci have an exceptional ability to adhere to the endocardium of the heart, great vessels, and valves.

In the present study, although bacterial growth was detected in postbrushing culture bottles of 7 out of 100 patients, the intensity of this bacteremia seemed to be very low grade as no bacterial colonies were formed in the pour-plate cultures belonging to these subjects. It was shown in animal experiments that 10^3 to 10^9 colony forming units of bacteria per milliliter of blood was required to induce infective endocarditis.[6]

In the studies on bacteremia related to toothbrushing in orthodontic patients by Chung et al.[12] and Schlein et al.[13] subjects used toothbrush without toothpaste and none used chlorhexidine or any antiseptic before toothbrushing. In the present study, the control group performed as in the previous studies, but in an effort to simulate the normal situation of the patients, the subjects in the third group were asked to brush their teeth with commonly used toothpaste. In order to search the possibilities for decreasing the number of oral bacteria and thus the risk of bacteremia, the patients in the second group were asked to rinse with 0.2% chlorhexidine gluconate before toothbrushing. Since chlorhexidine gluconate could have side effects such as selection of resistant bacterial strains with prolonged use[43,44] we also preferred to assess the efficacy of a toothpaste with phytochemicals like tea tree oil, peppermint oil, clove oil and bisabolol, in decreasing the risk of bacteremia. The most well known among these phytochemicals is tea tree oil, the antibacterial efficacy of which had been documented in numerous studies. In vitro susceptibility assays have shown that for all *Streptococcus species*, minimum inhibitory concentrations of tea tree oil ranged from 0.12 to 2% and minimum bactericidal concentrations ranged from 0.5 to 2%.[43] Time kill studies with *Streptococcus mutans* and *Lactobacillus rhamnosus* demonstrated that treatment with 0.5% tea tree oil reduced the number of viable organisms by $>$ 3 log within 30 seconds, and no viable organisms were detected after 5 minutes.[43] According to Takarada et al.,[27] exposure for 30 seconds to 0.5% tea tree oil killed *S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* completely. They claimed that tea tree oil completely killed gram-negative bacterial strains tested, even at a 0.2% concentration. They reported that tea tree oil had a marked adhesion-inhibiting effect on *S. mutans* and *P. gingivalis*, and suggested that these essential oils may also suppress biofilm formation. Filoche et al.[44] reported that tea tree oil demonstrated an inhibitory effect against both planktonic and biofilm growth of *S. mutans* at a concentration of 10 mg/mL. The antibacterial properties of tea-tree oil have been attributed to terpinen-4-ol, which is thought to damage cell membrane structures, and cause inhibition of membrane-bound enzymes. Based on these reports, the 0.35% concentration of tea tree oil available in One Drop Only toothpaste is expected to provide a substantial antibacterial effect. Peppermint oil has also been shown to be a potent antibacterial agent against oral bacteria.[45] Antibacterial effects of clove oil and bisabolol against oral pathogens have not been documented yet. The promising *in vitro* results obtained with tea tree oil need to be supported with clinical trials that will assess *in vivo* efficacy and determine appropriate concentrations for oral use. Safety

issues associated with oral use of essential oils need to be investigated, too.

Comparison of the findings obtained from the four groups did not reveal statistically significant differences. However, although not significant, the decrease in the occurrence of bacteremia in the second and fourth groups leads one to think that the question of effectiveness of antibacterial agents used prior to or during toothbrushing may deserve further investigation. A similar study conducted on a larger sample may provide more significant results because as sample size increases, the power to detect an actual difference also increases. Unfortunately, the difficulty in finding enough number of volunteering and suitable patients is a factor, which can limit sample size in bacteremia studies.

CONCLUSIONS

Toothbrushing in orthodontic patients yielded to an increase in the occurrence rate of bacteremia when using normal toothpaste or no toothpaste at all.

The use of chlorhexidine mouthwash before toothbrushing, and brushing with antimicrobial toothpaste did not show a statistically significant difference in preventing occurrence of bacteremia.

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