Original Article

Microbiological Evaluation of 0.2% Chlorhexidine Gluconate Mouth Rinse in Orthodontic Patients

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ABSTRACT

Objective: To assess the effectiveness of 0.2% chlorhexidine gluconate mouth rinse on *Streptococcus mutans* and lactobacilli in orthodontic patients with fixed appliances.

Materials and Methods: Twenty patients, aged 13–18, with fixed orthodontic appliances participated in the study. The levels of *S mutans* and lactobacilli in saliva samples were evaluated at four stages: at the beginning of the orthodontic treatment, at least 2 weeks after the bonding of brackets, 1 week after the introduction of 0.2% chlorhexidine gluconate mouth rinse, and at the fourth week. The changes in *S mutans* and lactobacilli levels were analyzed via Wilcoxon test.

Results: Increases in bacterial levels of *S mutans* and lactobacilli were detected after the orthodontic appliances were bonded. A significant decrease in *S mutans* levels was observed 1 week after the introduction of chlorhexidine mouth rinse.

Conclusions: An 0.2% chlorhexidine gluconate mouth rinse decreased *S mutans* levels, but had no effect on lactobacilli levels.

KEY WORDS: 0.2% Chlorhexidine gluconate; *S mutans*; Lactobacilli

INTRODUCTION

Mechanical tooth-cleaning is very important for patients with fixed orthodontic appliances. Malocclusions and fixed orthodontic appliances cause difficulties in brushing and increase the accumulation of microbial plaque. This, in turn, facilitates the formation of dental caries and induces periodontal problems, with deterioration of the ecologic balance of the oral flora.[1,2]

Several studies have reported that there is a positive correlation between dental caries and the degree of infection with *Streptococcus mutans* and lactobacilli.[3–5] According to recent research, it was found that the number of *S mutans* in saliva is higher than lactobacilli counts in 10-year-old children.[6]

Orthodontic appliances also reduce the effect of brushing on plaque and salivary flow. Increased levels of *S mutans* and lactobacilli are detected in the oral cavity after the bonding of orthodontic attachments.[7] Furthermore, metallic brackets have been found to make specific changes in the oral environment, such as a decrease in pH and affinity of bacteria to a metallic surface because of electrostatic reactions.[8]

The mouth rinse, as a chemical agent, could be a useful clinical adjunct for reducing the bacterial plaque accumulation during the active phase of orthodontic treatment. Such chemical agents also help orthodontic patients who have difficulties in maintaining plaque control by mechanical means alone. These patients should be reminded that chemical agents are not substitutes for thorough brushing and interproximal cleaning.[9–13] In addition to mechanical tooth-cleaning, any chemical mouth rinse could be recommended to orthodontic patients.

Chlorhexidine is an agent that is frequently used against *S mutans*. It is commercially available in the forms of mouth rinse, gel, and varnish. The purpose of this study was to investigate the effect of 0.2% chlorhexidine gluconate mouth rinse on *S mutans* and lactobacilli in orthodontic patients with fixed appliances.

MATERIALS AND METHODS

Twenty male patients with fixed appliances were selected for this study from the Department of Orthodontics at Kasimpasa Military Hospital (Istanbul, Turkey). The ages of the patients varied from 13 to 18 years.

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TABLE 1. Comparison of *Streptococcus mutans* and Lactobacilli Levels (CFU [log]/mL) at Different Stages with Respect to Stage 0 ($P < .05$)[a]

					P		
Bacteria	Stage 0	Stage I	Stage II	Stage III	Stage 0–Stage I	Stage 0–Stage III	Stage 0–Stage II
S mutans	85 ± 23.7	460 ± 44.7	182 ± 29.3	91.5 ± 24.9	.0001*	.212	.0001*
Lactobacilli	9.3 ± 5.8	34.0 ± 14.3	31.7 ± 11.2	31.2 ± 9.16	.0001	.0001	.0001

[a] For each group, n = 20 and significance value $\alpha = .05$. Values are mean ± SD.
* $P < .001$.

The following criteria were used in selecting the patients:

- They were undergoing full-banded edgewise extraction treatment with brackets on their anterior teeth and bands on their molars.
- They were at least 13 and no more than 18 years old.
- There was no evidence of decalcification on their teeth.
- There was no known hypersensitivity to chlorhexidine.
- There was no known medical problem or evidence of current antibiotic therapy.
- No anterior composites were present.

Each patient was provided with an Oral-B Advantage toothbrush and Ipana toothpaste (Procter & Gamble Ltd, Cincinnati, Ohio). Patients were all instructed to brush for a minimum of 3 minutes once in the morning after breakfast and once in the evening before bedtime. The mouth rinses were used by patients according to the manufacturer's directions after toothbrushing according to the following regimen: 0.5 ounces of 0.2% chlorhexidine gluconate was applied for 30 seconds after breakfast and before bedtime. The patients were instructed not to take any liquid or food into their mouths for at least 30 minutes after using the prescribed mouth rinse. Samples of stimulated saliva were taken by giving the patient a piece of paraffin to chew for 5 minutes until 3 mL of saliva was collected. The saliva was kept on ice until used.

Saliva samples were taken at four stages in the study:

- Stage 0: The first sample was taken immediately after the orthodontic appliances were bonded. After the first sample was taken, the patient received oral hygiene instruction.
- Stage I: The second sample was taken after 2 weeks. In that period of time, possible changes in the bacterial flora were allowed. The patients were instructed to use chlorhexidine mouth rinse starting from the second week.
- Stage II: At the end of the third week, the third sample was taken. Note that the third saliva sample was taken after 1 week of mouth rinse usage.
- Stage III: The fourth saliva sample was taken at the end of 4 weeks. The patients had used chlorhexidine gluconate mouth rinse for the preceding 2 weeks.

The saliva samples were mixed on a vortex for 1 minute. After agitation, the samples were diluted in phosphate buffer to 10^{-1}, 10^{-2}, and 10^{-3}. From each of the dilutions, 25 μL was spotted in duplicate on one-third of the surface of an agar plate. Mitis Salivarius agar (B298, Difco, Detroit, Mich) with the addition of sucrose and bacitracin was used for the culture and detection of *S mutans*. Rogosa SL agar (B480, Difco) was used to determine the levels of lactobacilli. The quantitative estimation of *S mutans* and lactobacilli was carried out according to a micromethod described by Westergen and Krasse.[14] Results are expressed as colony-forming units (CFU)/mL.

Results are reported as the mean of four different readings. The data were analyzed according to the nonparametric Wilcoxon signed rank test (SPSS software, SPSS, Chicago, III). Statistical significance level was determined at $P < .05$.

RESULTS

S mutans levels varied between 30.1 and 500 CFU (log)/mL and lactobacilli levels varied between 5.6 and 50.9 CFU (log)/mL over the experimental period.

The results are summarized in Tables 1 and 2. Table 1 presents a comparison of stage 0 with the other stages, and Table 2 gives the comparison of stage I with stages II and III. Table 1 shows that the *S mutans* level was 85 CFU (log)/mL and the lactobacilli level was 9.3 CFU (log)/mL at baseline. These findings were significantly increased to 460 CFU (log)/mL ($P = .0001$) for *S mutans* and 34 CFU (log)/mL ($P = .0001$) for lactobacilli after bonding the fixed appliances. At stage II, the *S mutans* level was significantly decreased to 182 CFU (log)/mL ($P = .0001$). Moreover, we observed a decrease in the lactobacilli level to 31.7 CFU (log)/mL ($P = .0001$) after chlorhexidine introduction. At stage III, the *S mutans* level was 91.5 CFU (log)/mL ($P = .212$); however, the decrease at stage III was not statistically significant ($P > .05$). Furthermore, at stage III, the increase in the lactobacilli level

to 31.2 CFU (log)/mL ($P = .0001$), compared with baseline values, was statistically significant ($P < .05$) (Table 1).

On the other hand, Table 2 shows that the decreases in *S mutans* levels between stages I and II, I and III, and II and III are statistically significant ($P = .0001$). However, we can not claim that there are statistically significant differences between lactobacilli levels at stages I and II, I and III, or II and III ($P > .05$; Table 2).

DISCUSSION

During orthodontic treatment, practicing satisfactory oral hygiene is a difficult task for orthodontic patients because of brackets and wires. Failure to maintain proper oral hygiene leads to tooth damage. Therefore, levels of cariogenic pathogens should be constantly reduced during the active phase of orthodontic treatment if a chemical agent can be used.[15,16] This study evaluated the effects of 0.2% chlorhexidine gluconate on *S mutans* and lactobacilli levels.

In some previous studies, it has been suggested that higher concentrations of antimicrobial agents and multiple treatments extend the time of effectiveness against *S mutans*. In contrast to these studies, it was found in our study that as low a concentration as 0.2% chlorhexidine gluconate mouth rinse significantly reduced the *S mutans* level.[12,17] However, Zanella et al[18] investigated the influence of 0.12% chlorhexidine gluconate and 0.2% chlorhexidine digluconate on both dental plaque accumulation and salivary *S mutans* and showed that there is no significant difference between them.

The results of our study have shown that *S mutans* and lactobacilli levels were significantly increased after bonding the fixed appliances. However, the *S mutans* levels significantly decreased after the administration of 0.2% chlorhexidine gluconate. These findings confirm those of Beyth et al.[11] Our result of significant reduction in *S mutans* levels is similar to the results of studies in which chlorhexidine varnish is used.[11–13]

It should be noted that 0.2% chlorhexidine gluconate had no effect on the incidence of lactobacilli levels. This is probably because of the fact that lactobacilli, in contrast to *S mutans*, have a low sensitivity to chlorhexidine. This result agrees with other chlorhexidine clinical tests.[13,19]

Attin et al[20] reported that varnishes with high concentrations of chlorhexidine (40% chlorhexidine and Cervitec) revealed a significantly stronger reduction of *S mutans* in plaque and saliva compared to low-concentration varnish during a 2-week period. However, they could not observe any reduction in lactobacilli count with high-concentration chlorhexidine varnish usage in the patient. Furthermore, de Soet et al[21] studied the effect of 40% chlorhexidine varnish during a 30-month period. They also found that 40% chlorhexidine varnish did not decrease the number of cariogenic bacteria. It could be concluded that chlorhexidine varnish in any concentration and period cannot affect the level of lactobacilli.

The number of lactobacilli and *S mutans* in the saliva is a sign of a cariogenic diet. These bacteria can rapidly metabolize dietary sugars to acid, creating a local low pH. These organisms grow and metabolize optimally at a low pH.[22,23] It has been reported that decreases in sucrose intake decrease *S mutans* and lactobacilli number in plaque and saliva.[22] That is why decreasing the consumption of sugar and sugar-containing products could increase the effectiveness of chlorhexidine applications. Furthermore, Juric et al[24] investigated the effect of different caries-preventive agents (aminfluoride solution, Proxyt paste, chewing gum containing xylitol and fluoride, and chlorhexidine solution) on salivary *S mutans* and lactobacilli. They observed that professional tooth-cleaning and the usage of chewing gum with xylitol and fluoride on a daily basis could be helpful in reducing cariogenic bacteria.

The germicidal effect of fluoride on cariogenic bacteria (such as *S mutans* and lactobacilli) is the inhibition of glycolysis. In addition, fluoride acidifies the interior of cells and inactivates some enzymatic metabolic processes.[25] Ahumado Ostenga et al[26] showed that the effects of sodium fluoride (NaF) and chlorhexidine mouth rinse with different concentrations changed depending on the species of lactobacilli. They reported an inhibition of lactobacilli by NaF of between 57% and 84% at 20 mmol·L^{-1}, whereas high concentrations of chlorhexidine (197 and 98 mmol·L^{-1})

TABLE 2. Statistical Evaluation of *Streptococcus mutans* and Lactobacilli Levels (CFU [log]/mL) Between Stages I and II, Stages I and III, and Stages II and III[a]

				P		
Bacteria	Stage I	Stage II	Stage III	Stage I–Stage II	Stage I–Stage III	Stage II–Stage III
S mutans	460 ± 44.7	182 ± 29.3	91.5 ± 24.9	.0001*	.0001*	.0001*
Lactobacilli	34 ± 14.3	31.7 ± 11.2	31.2 ± 9.16	.095	.197	.617

[a] For each group, n = 20 and significance value $\alpha = .05$. Values are mean ± SD.
* $P < .001$.

showed a complete inhibitory effect on *Lactobacillus salivarius* CRL1414 and *L plantarum* 1363. However, they did not observe any inhibition of *L plantarum* 1356.

Based on the previous studies, we can conclude that *Lactobacillus* species are sensitive to high concentrations of chlorhexidine mouth rinse rather than to high concentrations of chlorhexidine varnish. Furthermore, some species of *Lactobacillus* are more sensitive to chlorhexidine, whereas some of them are less sensitive. The analysis of the effect of 0.2% chlorhexidine gluconate mouth rinse for longer periods remains for future research.

CONCLUSIONS

- In combination with mechanical plaque removal, 0.2% chlorhexidine gluconate is an important therapeutic agent in controlling the *S mutans* and lactobacilli levels of orthodontic patients with fixed appliances.
- *S mutans* levels decreased significantly whereas lactobacilli levels remained the same.
- Patients can successfully use 0.2% chlorhexidine gluconate mouth rinse after toothbrushing every day once in the morning after breakfast and once in the evening before bedtime in order to decrease *S mutans* levels.

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