Effects of probiotics on salivary *Streptococcus mutans* and *Lactobacillus* levels in orthodontic patients

Sevtap Alp and Zeliha Müge Baka
Konya, Turkey

Introduction: In this study, we aimed to determine the effect of regular probiotic consumption on microbial colonization in saliva in orthodontic patients and to comparatively evaluate the difference between the systemic consumption of probiotic products and the local application. **Methods:** This study included 3 groups with 15 orthodontic patients in each. The control group included patients who had no probiotic treatment, the subjects in the kefir group consumed 2 × 100 ml of kefir (Atatürk Orman Ciftligi, Ankara, Turkey) per day, and the subjects in the toothpaste group brushed their teeth with toothpaste with probiotic content (GD toothpaste; Dental Asia Manufacturing, Shah Alam, Selangor, Malaysia) twice a day. Samples were collected at 3 times: beginning of the study, 3 weeks later, and 6 weeks later. The salivary flow rate, buffer capacity, and *Streptococcus mutans* and *Lactobacillus* levels in the saliva were evaluated. Chair-side kits were used to determine the *S mutans* and *Lactobacillus* levels. **Results:** A statistically significant decrease was observed in the salivary *S mutans* and *Lactobacillus* levels in the kefir and toothpaste groups compared with the control group ($P < 0.05$). A statistically significant increase was observed in the toothpaste group compared with the control and kefir groups in buffer capacity. Changes in the salivary flow rate were not statistically significant. **Conclusions:** The regular use of probiotics during fixed orthodontic treatment reduces the *S mutans* and *Lactobacillus* levels in the saliva. (Am J Orthod Dentofacial Orthop 2018;154:517-23)

Brackets, bands, and wires used in patients receiving fixed orthodontic treatment are convenient areas for the retention of food. Since oral hygiene becomes more difficult, an ecological environment facilitating the growth of microorganisms emerges and causes tooth decay.[1-3]

Streptococcus mutans and *Lactobacillus* are the main microorganisms that cause tooth decay. *S mutans* is responsible for the initial decay.[4] It is found in microbial dental plaque and saliva in high quantities in patients with a high risk of decay, and it has a positive correlation with decay activity. *Lactobacillus* plays a role in progressive decay lesions.[5]

From the Department of Orthodontics, Faculty of Dentistry, Selçuk University, Konya, Turkey.
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Address correspondence to: Zeliha Müge Baka, Selçuk Üniversitesi, Dişhekimliği Fakültesi, Ortodonti AD, Selçuklu-42079, Kampüs/Konya, Turkey; e-mail, mugen97@hotmail.com.
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One of the most troubling side effects of orthodontic treatment is white spot lesions, which have been mitigated by topical fluoride applications. Fluoride varnishes, toothpastes, and sealants are used for this purpose. Fluoride-releasing elastomeric chains, glass ionomer cements, and composites have been developed for long treatment periods in orthodontic patients. Many methods, including antimicrobial and antibiotic treatments, have been adopted, but their effectiveness can only be observed when they are regularly used. This disadvantage leads to the idea of using probiotics as an alternative.[6,7]

Probiotics are bacterial cultures or living microorganisms that positively affect general health when taken in sufficient quantities through nutrition.[8,9] Probiotics work as antagonists of cariogenic bacteria and prevent their multiplication; consequently, the acid formed by sugar metabolism is neutralized.[8]

The number of dairy products containing probiotics is increasing. These include milk, yogurt, cheese, and ice cream.[10] Most of the probiotic products on the market contain at least 1 probiotic bacterium.[11] Kefir is a milk fermentation product consisting of yeast and fungi as well as probiotic bacteria.[12,13] Its health benefits include calcium, amino acids, folic acid, and vitamins K, B1, and B2.[14]

Studies demonstrating the effect of probiotic products in orthodontic patients[7,15,16] and comparing probiotic systems[7] are limited. No study has evaluated the effect of kefir on orthodontic patients. The hypotheses that we investigated were that (1) the use of probiotic products in orthodontic patients reduces salivary *S mutans* and *Lactobacillus* levels, and (2) there is a difference in the effect of reducing salivary microbial colonization between systemic consumption and local application of probiotic products. Therefore, in this study, we aimed to determine the effect of regular probiotic use on salivary microbial colonization in patients receiving fixed orthodontic treatment and to comparatively evaluate the difference between the systemic consumption and the local application of probiotic products.

MATERIAL AND METHODS

A priori power analysis was performed by G*Power software (version 3.0.10; Franz Faul, Universitat Kiel, Kiel, Germany). Based on the 1:1 ratio between groups, a total sample size of 45 participants would give more than 80% power (actual power, 0.8173) for repeated measures analysis of variance with 3 groups and 3 time points to detect significant differences with a 0.40 effect size at the $\alpha = 0.05$ significance level. Forty-five patients (27 girls, 18 boys) who applied for orthodontic treatment at the Department of Orthodontics of the Faculty of Dentistry at Selçuk University in Konya, Turkey, were included in our study. Their average age was 14.43 ± 1.93 years (range, 12-17 years). Approval for the study was obtained from the ethics committee presidency (2014/34) of the Faculty of Medicine at Selçuk University. All patients and their parents were informed about the research and signed informed consent forms. The criteria for inclusion in the study were mild or moderate crowding; nonextraction treatment; good oral hygiene; good general health; permanent dentition; no use of anti-inflammatory medications or antibiotics within the last month; no use of products with xylitol, fluoride, or probiotic content; and fewer than 5 decayed, missing, or filled teeth.

Before the study, all patients were given oral hygiene training and asked to brush their teeth twice a day. To ensure standardization, we provided the toothpaste and toothbrushes. Forty-five subjects were randomly divided into 3 groups of 15. Randomization was stratified according to sex and age. The control group consisted of patients who received no probiotic treatment. Those in the kefir group consumed 2 × 100 ml of kefir (Atatürk Orman Ciftligi, Ankara, Turkey) per day. Kefir contains a mixture of lactic acid bacteria culture (*Lactococcus lactis* subsp, *Leuconostoc* sp, *Lactobacillus* sp, and *S thermophilus*) and yeasts isolated from kefir grains. The subjects in the toothpaste group brushed their teeth with toothpaste with probiotic content (GD toothpaste; Dental Asia Manufacturing, Shah Alam, Selangor, Malaysia) twice a day in the morning and evening. GD toothpaste contains bacteriocin extracted from lactic acid bacteria, a probiotic bacterium. These patients did not use normal toothpaste during the study. To ensure the regular use of probiotic toothpaste and kefir, the patients were given daily checklists and asked to mark after each use. In addition, the patients were regularly called to check their use of the products. Due to the differences in the probiotic products used, blinding of either patient or operator was not possible. Blinding was applicable for outcome assessment only.

Mandibular teeth were bonded 4 weeks after the bonding of the maxillary teeth in all patients. The study began 3 months after the maxillary teeth were bonded. No materials—eg, elastic ligatures, chains, coil springs, or figure-eight ligatures that could adversely affect oral hygiene—were used before and during the study. Samples were taken at the beginning of the study, 3 weeks later, and 6 weeks later. The patients were warned not to eat or drink anything at least 2 hours before the saliva samples would be taken.

Stimulated saliva samples were taken between 9:00 and 10:00, and the salivary flow rate, buffering capacity, salivary *S mutans* and *Lactobacillus* levels were evaluated. The patients were asked to chew paraffin gum for 5 minutes and to collect their saliva using a test tube. The salivary flow rate was calculated in minutes per milliliter. Buffering capacity was determined using the CRT buffer (Ivoclar Vivadent, Schaan, Liechtenstein) according to the manufacturer's instructions. Blue color indicated high (pH ≥6), green color indicated medium (pH 4.5-5.5), and yellow color indicated low (pH ≤4.5) buffering capacity.

The CRT bacteria (Ivoclar Vivadent) was used to determine *S mutans* and *Lactobacillus* levels in saliva. The special medium for *S mutans* and *Lactobacillus* was moistened with saliva. Then the sodium bicarbonate tablet was added to the tube and incubated at 37°C for 48 hours. Scores were calculated by comparing the densities of *S mutans* and *Lactobacillus* colonies formed on the medium, with the schemes specified by the manufacturing company.

Statistical analysis

Data were analyzed with the SPSS statistical package (version 22.0; IBM, Armonk, NY). The Shapiro-Wilk test