Systemic consumption of probiotic curd and use of probiotic toothpaste to reduce *Streptococcus mutans* in plaque around orthodontic brackets

**Introduction:** The objectives of the study were to evaluate and compare the effects of the systemic consumption of probiotic curd and the topical application of probiotic toothpaste on the *Streptococcus mutans* levels in the plaque of orthodontic patients. **Methods:** The study consisted of 60 orthodontic patients divided into 3 groups of 20 each. Group 1 was the control group. The patients in group 2 were given probiotic curd, and those in group 3 were asked to brush twice daily with probiotic toothpaste (GD toothpaste; Dental Asia Manufacturing, Shah Alam, Selangor, Malaysia). Samples were collected at 2 times: before the study began and after 30 days. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors using a 4-pass technique. The presence of *S mutans* was evaluated using real-time polymerase chain reaction. Statistical analysis was performed, and comparisons were made using a 2-tailed chi-square test for categorical data (*P* < 0.05). **Results:** At the end of the study, there were reductions in *S mutans* concentration in groups 2 and 3 that were statistically significant compared with group 1, but there was no statistically significant difference between groups 2 and 3. **Conclusions:** The consumption of probiotic curd and the use of probiotic toothpaste cause a significant decrease in the *S mutans* levels in the plaque around brackets in orthodontic patients. Although the probiotic toothpaste was more effective than systemic consumption, this was not statistically significant. (Am J Orthod Dentofacial Orthop 2013;144:67-72)
Biological methods such as antibiotics, antimicrobial therapy with chlorhexidine, povidone iodine, fluoride, and penicillin have gained importance in recent years. The application of broad-spectrum antibiotics and antimicrobial therapy can suppress the caries infection but never totally eliminate it. None of these medications has been able to successfully preclude the regrowth of residual pathogens or reinfection from external sources; this means that antibiotic and antimicrobial therapies must be given at regular intervals for effective long-term results.

At the turn of the 20th century, Elie Metchnikoff, a Nobel Prize-winning Russian, made the revolutionary discovery of probiotics. Probiotics are “live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance.” Lactic acid bacteria and bifidobacteria are the most common types of microbes used as probiotics, but certain yeasts and bacilli can also be helpful. They act by competitively inhibiting the pathogenic bacteria because they have greater adhesion to the tissues. They inhibit pathogens but do not inhibit friendly bacteria. Studies have shown that once the pathogenic organisms are replaced the reintroduction of the pathogen does not occur easily.

Probiotics are commonly consumed as part of the diet in several cultures in the form of fermented foods such as yogurt and soy yogurt, or as dietary supplements with added active live cultures. They have proved to be beneficial in treating malnourishment, lactose intolerance, calcium availability, bowel problems such as constipation, urogenital infections, and atopic diseases such as antibiotic-induced diarrhoea, and in improving the immune system, alleviating chronic intestinal inflammatory diseases, and preventing and treating pathogen-induced diarrhea.

A few studies have evaluated the effects of local administration of probiotic agents such as mouthwashes, lozenges, tablets, straws, milk, cheese, ice cream, chewing gums, yogurt, and other supplements and have found that these have a beneficial effect on oral health. The benefits on oral health in preventing gingivitis, halitosis, and caries have been recognized, and thus probiotics have been incorporated into mouthwashes and dentifrices for popular consumption. Some studies have established that the level of \textit{S. mutans} in saliva is reduced after the use of probiotics; this would be beneficial in orthodontic patients also.

However, there are few studies in the literature on the effects of probiotics in orthodontic patients, since their use in our specialty is still in an infantile stage. \textit{S. mutans} concentration in plaque would be more representative of the caries-inducing potential in the anterior teeth where salivary clearance is less effective. Since the localized effect of probiotics on the plaque surrounding orthodontic brackets has not been studied, we conceived this study to evaluate whether probiotic systems are beneficial to orthodontic patients. It is desirable to establish which delivery system is more efficient, and thus this study was designed to compare the efficacy of systemic ingestion and topical applications.

Our aims were to evaluate and compare the effect of probiotic systems (systemic and local) on the \textit{S. mutans} levels in the plaque surrounding brackets in orthodontic patients.

**MATERIAL AND METHODS**

The study was double blinded and randomized, consisting of 60 randomly selected patients having orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics at Sri Ramachandra University in India. The following enrollment criteria were used: (1) orthodontic treatment with the straight wire appliance (MBT, 0.022-in slot; 3M Unitek, Monrovia, Calif), (2) permanent dentition, (3) good general health (no significant medical history or drug use during the last month), (4) no anti-inflammatory or antibiotic medications taken in the month before the study, (5) no chewing gum or mouthwash used in the last week and during the study, (6) habit of brushing twice daily with fluoride toothpaste, and (7) age between 14 and 29 years (average, 20 years).

All subjects had good oral health with no open or untreated caries lesions or gingival inflammation, and they claimed to have daily tooth brushing habits. There were 42 female and 18 male subjects. They were divided into 3 groups of 20 each. Group 1 consisted of patients who received no probiotic treatment (control group). The patients in group 2 were given 200 mg of probiotic curd (Active Plus; Nestle, Chennai, India), instructed to eat it with their lunch for 30 days, and asked to brush twice daily with their regular fluoride toothpaste (Colgate Strong Teeth; Colgate-Palmolive Ltd, Solan, Himachal Pradesh, India). The patients in group 3 were asked to brush twice daily with probiotic toothpaste (GD; Dental Asia Manufacturing, Shah Alam, Selangor, Malaysia) only for 30 days and to discontinue using their normal toothpaste. The patients were asked to brush with an up-and-down motion on the front teeth and a circular motion on the back teeth for 2 minutes; this was demonstrated by the same operator (J.E.J.).

The patients were instructed to avoid chewing gums, mouthwashes, and antibiotics during the study. Samples were collected at 2 times: before the study began and after 30 days.

At each time interval, the elastomeric modules (Ormco, Orange, Calif) were carefully removed to
disengage the archwires by the same operator. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors with a sterilized scaler using a 4-pass technique as suggested by Pellegrini et al.20 Four passes, each along the tooth at the bracket interface at the gingival, mesial, distal, and occlusal aspects, were used to prevent overloading the instrument tip.

The samples were placed into individual micropipette tubes with anonymous coding and sealed for transport for DNA isolation to the Medox Biotech India laboratory in Chennai, India. The coding of the specimens was not disclosed to the laboratory personnel and helped to minimize experimental bias.

The ultrapure genomic DNA Spin Miniprep (Medox Biotech India) kit was used for fast isolation of genomic DNA. The quality of DNA thus obtained was measured using a nano-drop technique in the Department of Bio-medicine of Sri Ramachandra University. The primers and probes specific for S mutans were manufactured by Bangalore Genie (Shushruti Nagar, Bangalore, India). The oligonucleotide primers used were Sm F5 5'-AGC CAT GCC CAA TCA ACA GGT T and Sm R4 5'CGC AAC ATC TTG ATC AG. The samples were then taken to the Central Research Foundation, Sri Ramachandra University, where the real-time polymerase chain reaction was done (model 7900HT; Applied Biosystems, Invitrogen BioServices India Pvt Ltd, Whitefield, Bangalore, India) using SYBR green assay for relative quantification of the bacteria in the samples.

The polymerase chain reaction values were obtained in the form of a graph that was interpreted with the software from Applied Biosystems. The values were tabulated, and the statistical analysis was performed with SPSS software (version 2; SPSS, Chicago, Ill). Comparisons of the concentration between groups 1 and 2, and also comparisons among the 3 groups, were done using a 2-tailed chi-square test for categorical data. P < 0.05 was considered statistically significant.

In a real-time polymerase chain reaction assay, a positive reaction is detected by accumulation of a fluorescent signal. The cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold. Real-time assays undergo 40 cycles of amplification. If the sample does not reach this level even after 40 cycles, then it does not contain the organism and is called “undetermined.” The real-time polymerase chain reaction shows the relative quantification of the bacteria in the samples. The quantification is done by evaluating the cycle threshold values, which are the threshold values at which there is expression of the bacterial genome. The cycle threshold value is inversely proportional to the amount of bacterial genome present. An increase in cycle threshold values indicates a decrease in bacteria, and a decrease in cycle threshold values indicates an increase in bacteria.

RESULTS

Before the study began, 16 samples (80%) showed genomic expression in group 1, 15 samples (75%) showed genomic expression in group 2, and 14 samples (70%) showed genomic expression in group 3 (Table I).

There was no statistically significant difference in the S mutans concentrations among the 3 groups before the study (P > 0.05).

After 30 days, 15 samples (93.75%) showed genomic expression in group 1, 9 samples (60%) showed genomic expression in group 2, and 8 samples (57%) showed genomic expression in group 3. Five samples in group 1, 11 samples in group 2, and 12 samples in group 3 were genetically indeterminate (Table I) (calculated by cycle threshold values greater than 40).

In group 1, 18% (3 of 16) of the samples showed a reduction in S mutans concentration, whereas in groups 2 and 3, all samples (15 of 15, and 14 of 14, respectively) showed reductions in S mutans concentrations (calculated by the increase in cycle threshold values). The samples with increases in the cycle threshold values after 30 days would have reductions in S mutans.

This showed no statistically significant reduction in S mutans in group 1, but there were statistically significant reductions in groups 2 and 3 (Tables I and II).

There were statistically significant differences in the reduction of S mutans concentration between groups 1 and 2, and groups 1 and 3, but no significant difference between groups 2 and 3 (Table II).

DISCUSSION

Enamel demineralization, or white spot lesions, a common esthetic hurdle in orthodontic treatment, is increased when a patient’s oral hygiene is poor. It is mainly caused by the organic acids produced by cariogenic microorganisms in the oral cavity. The prevalence of white spot lesions was reported to be as high as 50% in some patients.2 The demineralization of enamel, a precursor of the carious lesion, appears opaque because of the decreased light-scattering ability of the decalciﬁed enamel.

In the anterior region, the lateral incisors are the most susceptible because of decreased salivary clearance and also less space between the bracket and gingiva.21 This creates less accessibility for oral hygiene techniques. White spot lesions in the anterior region are clearly visible and are a major esthetic concern.

Among the cariogenic microorganisms, the initiation of carious lesions is mainly due to mutans streptococci
called bacteriocins: eg, reuterin.10,27 The ef
probiotic species also secrete antimicrobial compounds
spaces where the pathogens would invade. 19 Some
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and bi
The probiotic organisms, lactobacillus, streptococci,
the bacterial pathogens off oral tissues by
lining for oral tissues against oral diseases by keeping
Probiotics can create a bio
the microbial
20th century. Probiotics have been used in modifying
major side effects.
These disadvantages have caused probiotics to be
only as long as they are supplied at regular intervals.5
therapies, have been tried, but their ef
methods, including antimicrobial and antibiotic
and composites for long treatment periods. Many other
releasing elastomeric chains, glass ionomer cement,
and after 30 days (T2)
Table II. Cycle threshold values before the study (T1)
and after 30 days (T2)
Table I. Reduction in S mutans levels after 30 days
debacterization of probiotics in reducing
S mutans.
A study in which the effect of probiotic yogurt was
evaluated on S mutans counts in the saliva of orthodon-
tic patients showed that the number of subjects with high S mutans counts decreased from 63% to 21% after 2
weeks of consumption.16 We chose to evaluate the
plaque around orthodontic brackets because it would be a more accurate measure of the S mutans that cause
white spot lesions.
Many studies have used saliva to determine the
amounts of S mutans in the oral cavity.8-10,16,17 The major disadvantage of using saliva is that the S mutans in the saliva is a total count of the
organisms in the oral cavity from previous carious
lesions, the tongue, and other sites that harbor the
organisms, and it is not specific to the tooth surface. Some studies have shown a difference between the
salivary S mutans counts and the S mutans counts in
plaque.28,29 Since the anterior region has less salivary
clearance, plaque accumulation around orthodontic
brackets is a more specific region that harbors the
S mutans and can be considered more reliable in
regard to white spot lesions.
Polymerase chain reaction is a revolutionary
development in science because it helps to determine
even minute quantities of bacteria. Studies have shown high sensitivity and reliability of these machines in
detecting bacteria even to a 2-fold change in the
bacteria count. Few studies have evaluated the S mutans
levels with polymerase chain reaction. The previous
studies were based on chairside kits or other immuno-
logic and laboratory procedures.9,10,16,17 The results of
our study are similar to them and establish the ef
of probiotics in reducing S mutans levels using a more
precise and sensitive methodology: real-time polymer-
ase chain reaction.30
In our study, there were prevalences of S mutans of
80% in group 1, 75% in group 2, and 70% in group 3 before the study began. These values were similar to
earlier studies that showed prevalences greater than
60%.11,13,21,31 After 30 days, the prevalence of S mutans in group 1 remained the almost the same at
93.75%, but there were decreases in S mutans in

<table>
<thead>
<tr>
<th>Group</th>
<th>Cycle threshold value</th>
<th>T1</th>
<th>T2</th>
<th>Chi-square test</th>
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<tr>
<td>1</td>
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<td>4</td>
<td>5</td>
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<tr>
<td></td>
<td>&lt;40</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;40</td>
<td>5</td>
<td>11</td>
<td>0.05*</td>
</tr>
<tr>
<td></td>
<td>&lt;40</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;40</td>
<td>6</td>
<td>12</td>
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<tr>
<td></td>
<td>&lt;40</td>
<td>14</td>
<td>8</td>
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</table>

*P < 0.05 is statistically significant.

species such as S mutans, Streptococci sobrinus, and
Streptococci salivarius. S mutans are among the first
organisms to colonize the initial carious lesion. They are also the most cariogenic among the mutans
streptococci species because of their greater adhesive
properties and acid release.1,22-26
White spot lesions have been prevented over the years
using topical fluorides in the form of varnishes,
dentifrices, and sealants. They reduce the number and
size of white spot lesions. In orthodontics, continuous
fluoride release has been developed with fluoride-
releasing elastomeric chains, glass ionomer cement,
and composites for long treatment periods. Many other
methods, including antimicrobial and antibiotic
therapies, have been tried, but their efficiency lasts
only as long as they are supplied at regular intervals.5
These disadvantages have caused probiotics to be
considered an efficient alternative that could be
available in regular dietary supplements without causing
major side effects.
The use of probiotics has taken giant leaps since the
20th century. Probiotics have been used in modifying
the microbial flora of the stomach and intestines.8
Probiotics can create a biofilm, acting as a protective
lining for oral tissues against oral diseases by keeping
the bacterial pathogens off oral tissues by filling
the spaces where the pathogens would invade.19 Some
probiotic species also secrete antimicrobial compounds
called bacteriocins: eg, reuterin.10,27 The efficiency of
probiotics can be improved by the use of prebiotics.27
The probiotic organisms, lactobacillus, streptococci,
and bifidobacterium species, are genetically designed
to have greater adhesion and hence competitively inhibit S mutans.

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Group 1 (control)</th>
<th>Group 2 (curd)</th>
<th>Group 1 (control)</th>
<th>Group 3 (toothpaste)</th>
<th>Group 2 (curd)</th>
<th>Group 3 (toothpaste)</th>
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<tbody>
<tr>
<td>3</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>6</td>
<td>5</td>
<td>6</td>
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<td>No decrease</td>
<td>17</td>
<td>5</td>
<td>17</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Chi-square test</td>
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<td>0.000*</td>
<td>0.72</td>
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*P < 0.05 is statistically significant.
almost all subjects in groups 2 and 3; this was similar to the results of previous studies.8–13,16,17 After use of the probiotic curd and the toothpaste, almost all subjects in groups 2 and 3 showed a reduction in the *S mutans* levels; in some subjects, there were no detectable *S mutans* streptococci mutants after 30 days. This decrease was almost more than 2-fold in both groups 2 and 3.

An earlier study in orthodontic patients showed the efficiency of systemic consumption of probiotic yogurt for reducing the amount of *S mutans* in the saliva.16 Our study has shown more specific results with the systemic consumption of probiotic curd and the use of toothpaste on the plaque around orthodontic brackets. This reduction can be attributed to the formation of a biofilm that prevents adhesion of pathogens,19 the competitive inhibition of pathogenic bacteria, and the antimicrobial agents produced by the bacteria.10,29

Previous studies did not evaluate the topical effect of a probiotic toothpaste. Our study shows that the topical application of probiotic toothpaste was more efficient in reducing *S mutans* levels than probiotic curd, but this difference was not statistically significant. This study showed that probiotics can definitely be considered an option for the control of white spot lesions in orthodontic patients. Although both systemic and topical applications were effective, orthodontists would probably find introducing an oral hygiene regimen involving a probiotic dentifrice for their patients more practical during treatment.

**CONCLUSIONS**

The consumption of probiotic curd and the topical application of probiotic toothpaste caused significant decreases in the *S mutans* levels in the plaque around the brackets of orthodontic patients. This was a short-term study, and a longer period of evaluation would establish the long-term advantages of probiotics in orthodontic patients and the relative merits of systemic vs local probiotic therapy.

**REFERENCES**