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Original Article

Effect of Short-Term Placebo-Controlled Consumption of Probiotic Yoghurt and Indian Curd on the *Streptococcus mutans* Level in Children Undergoing Fixed Interceptive Orthodontic Therapy

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ABSTRACT

Objective: To examine the effect of short-term consumption of probiotic yoghurt, Indian curd, and ultra-heated yoghurt as placebo on the levels of salivary and plaque *Streptococcus mutans (S. mutans)* in children undergoing fixed interceptive orthodontic therapy.

Methods: A placebo-controlled double-blind study was carried out in a total of 30 children (8-15 years). The *S. mutans* level in the plaque and saliva were taken at the baseline and 2 weeks after the initiation of fixed orthodontic treatment by Dentocult SM kits. An equal number of participants randomized in three groups were asked to ingest 200 g of yoghurt containing *Lactobacillus acidophilus* La-1 and La-2 (>1×10^9 cfu/mL) once daily, Indian curd, or ultra-heated control yoghurt without viable bacteria and were followed for the *S. mutans* level after 2 weeks.

Results: A significant reduction in salivary *S. mutans* levels was recorded after probiotic yoghurt ingestion (p=0.001) in addition to a reduction in the plaque *S. mutans*, which was observed after Indian curd consumption (p=0.026).

Conclusion: Our findings suggest that short-term daily consumption of probiotic yoghurt along with Indian curd may help to reduce the levels of *S. mutans* in the saliva and plaque in children undergoing interceptive fixed orthodontic therapy.

Keywords: *Streptococcus mutans*, probiotics, yoghurt

INTRODUCTION

Insertion of fixed interceptive orthodontic devices with bands and brackets increases the caries risk, as such devices provide artificial niches for cariogenic microorganisms such as *Streptococcus mutans* (*S. mutans*) (1-3). The levels of cariogenic bacteria present in the plaque are higher in the cervical third of banded molars. A comprehensive management of dental caries involves the management of the disease as well as the lesion (4). There is now an intense focus on preventive strategies as minimal/noninvasive management. Considering the abovesaid, the use of probiotics is one of those strategies (5).

The current knowledge of the important role that the intestinal micro flora plays has promoted health by utilizing its microbial community. Therefore, preventive measures should concentrate on the change in oral ecology from pathogenic bacteria to ecofriendly bacteria in the oral cavity. This quest of low carcinogenicity has become focused recently on dairy products (6). The archetypical probiotic dairy products like yoghurt and Indian curd

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seem to be the most natural way to ingest probiotic bacteria (7). Indian curd is used in homes every day and is traditionally considered to be a great source of probiotic bacteria.

The goal of the study is to examine the effect of short-term consumption of probiotic yoghurt, Indian curd, and ultra-heated yoghurt as control on the levels of salivary *S. mutans* in pediatric orthodontic patients, which make a specific high-caries risk group, and whether incorporating these products in routine dietary practice is helpful in preventing a tooth structure damage due to orthodontic therapy.

METHODS

The study was double blinded and randomized consisting of 30 children (8-15 years in mixed/permanent dentition) having fixed interceptive orthodontic therapy. Enrollment criteria were the following: patients undergoing bilateral single-arch fixed interceptive and/or space management orthodontic therapy who had a good oral hygiene. Children were given tooth-brushing instructions and told to follow them twice a day using a regular fluoride tooth paste. The children were told to avoid chewing gums, candies, and medicated mouthwashes or to report immediately any medications that they have to use.

Clinical Methods

After explaining the study protocol and obtaining a written consent, 30 subjects (14 females, 16 males) undergoing orthodontic treatment were recruited and divided into three groups, 10 subjects each. Subjects who did not have clinically detectable caries and did not use systemic antibiotics 6 weeks prior to study were included. Ethical clearance was granted by the institutional ethical committee. Data were collected at three consecutive time periods. In Period I, before the banding for orthodontic therapy, oral prophylaxis was done, and instructions were given regarding the oral hygiene maintenance at the baseline examination. Period II started 2 weeks following the initiation of orthodontic treatment, and the aim was to see the effect of orthodontic treatment on the *S. mutans* count with the maintenance of oral hygiene. Period 3, a run-in period, started after 2 weeks of giving a masked cup (200 mg/day) of

I) Group (Py): probiotic yoghurt (Nestle Actiplus containing *Lactobacillus acidophilus* cfu/gm 20×10^7, *Bifidobacteria* 5.4×10^7 total useful bacteria 200,000,000)

II) Group (I c): Indian curd *Vita Lactobacillus acidophilus* cfu/gm$\times10^6$, *S. thermophiles* 35×10^4; total useful bacteria 5,350,000

III) Group (UHy): Nestle Actiplus ultra-heated (control) yoghurt

Microbiological Methods

A total of three saliva and plaque samples were obtained. Prior to sampling, subjects were instructed not to eat, drink, or brush their teeth for 1-2 hours, as it could affect results. Therefore, samples were collected between 10:00 am to 2:00 pm. Same banded teeth were selected in every patient for gingival plaque sampling, and they were isolated with cotton rolls and dried. A sterile explorer was carefully applied to buccal sites (cervical area) of the two molars to be banded. Stimulated saliva samples were collected after chewing a paraffin pellet of standard weight and size provided by the manufacturer (Dentocult SM kits) for 1 minute. The subjects were then asked to put two-thirds of the rough surface of the round tipped strip in the mouth and turn it around 10 times on the dorsal surface of the tongue. The strip was then removed by pulling between lightly closed lips to remove the excess saliva. Both square-tipped and round-tipped strips were clipped together, with their smooth surfaces facing each other, placed in the respective culture vial. These vials were then incubated in an upright position at the 37° centigrade for 48 hours, with the cap of each vial one-quarter of a turn open. To make subjects comfortable and accustomed to the sampling procedure, a mock trial was already carried out with wooden and plastic spoons on previous visit. We assessed the plaque and salivary S. mutans scores with a commercially available kit (Strip Mutans, Orion Diagnostica, Espoo, Finland) in accordance with the manufacturer's instructions (Jensen B., Bratithall D, 1989) (8).

After the incubation at the 37° centigrade for 48 hours, the presence of S. mutans was evidenced by dark-blue to light-blue, raised colonies on the rough surface of the strip. Density of the colonies on the rough surface of the round-tipped strip was compared with the interpretation chart in the manufacturer's manual, and accordingly a score of 0-3 was assigned (0, less than 104 CFU/mL, 1, less than 105 CFU/mL, 2, 105-106 CFU/mL, and 3, more than 106 CFU/mL).

A 7-day diet diary of each subject was also recorded as subjects were advised to take regular meals only, and it was also assessed for a total number of sugar exposures.

Statistical Analysis

We used the mean, median, and standard deviation for all bacterial parameters. To estimate the association between time-related variables (bacterial scores at the baseline, after 2 weeks of orthodontic treatment, and after 2 weeks of milk product consumption), the Wilcoxon signed-rank test was used. To see relations between variables, Fishers' exact test was used. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

The follow-up study population comprised of 27 subjects, with 3 subjects denied participation. The sample included 13 (48%) girls and 14 (52%) boys, with 12 (44%) aged 8-12 years and 15 (56%) aged 13-15 years (Table 1).

Table 1. Age distribution of subjects

	Subjects	
Age in Years	Number	Percentage
8 -12	12	44%
13-15	15	56%
Total	27	100%

Table 2. Salivary and plaque Streptococcus mutans scores at the baseline, after 2 weeks of orthodontic treatment and milk product ingestion after intake of Probiotic curd, Indian curd, and ultra-heated yoghurt. Probiotic yoghurt (Py), Indian curd (Ic), and ultra-heated yoghurt (Uhy) group; (A) baseline, (B) after 2 weeks of orthodontic treatment; (C) after milk product ingestion; Sal ms, salivary Streptococci mutans score; Pl ms, plaque *Streptococcus mutans* score
0, <104 CFU/mL; 1, <105 CFU/ml; 2, 105-106 CFU/mL; and 3, >106 CFU/mL

	Milk Product																	
	Probiotic yoghurt (Py) n						Indian curd (Ic) n						Ultra-heated yoghurt (Uti) n					
	A		B		C		A		B		C		A		B		C	
***Streptococcus mutans* score**	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms	Sal ms	Pl ms
0	2	-	-	-	5	2	3	-	-	-	3	3	-	-	-	-	-	1
1	2	-	2	1	4	-	2	3	1	1	1	2	1	1	-	-	-	-
2	4	2	3	4	-	7	3	2	4	6	3	4	3	6	5	8	5	6
3	1	1	4	-	-	-	1	-	4	-	2	-	5	-	3	-	3	-
4		6		4		-		4		2		-		2		-		1

Table 3a. Comparative evaluation (subject-wise) of salivary Streptococci mutans scores (As, at baseline; Bs, after 2 weeks of orthodontic treatment; Cs, 2 weeks after yoghurt consumption)

	MILK PRODUCT					
Streptococcus mutans	Probiotic yoghurt		Indian curd		Ultra-heated yoghurt	
score n=27	**As-Cs**	**Cs-Bs**	**As-Cs**	**Cs-Bs**	**As-Cs**	**Cs-Bs**
Decrease in score	7 p=0.0103*	9 p=0.001*	2	5	1	1
Increase in score	-	-	3	1	1	1
Same score	2	-	4	3	6	6

Table 3b. Comparative evaluation (subject-wise) of plaque Streptococus mutans scores (Ap, at the baseline; Bp, after 2 weeks of orthodontic treatment; Cp, 2 weeks after yoghurt consumption)

	MILK PRODUCT					
Streptococcus mutans	Probiotic yoghurt		Indian curd		Ultra-heated yoghurt	
score n=27	**Ap-Cp**	**Cp-Bp**	**Ap-Cp**	**Cp-Bp**	**Ap-Cp**	**Cp-Bp**
Increase in score	-	1	-	1	-	1
Same score	1	3	2	2	7	6

Salivary and plaque *S. mutans* scores were recorded in each subject from the probiotic yoghurt (Py), Indian curd (Ic), and ultra-heated yoghurt (UHy) groups at the baseline, after 2 weeks of orthodontic treatment and after 2 weeks of specified masked milk product ingestion. The baseline salivary *S. mutans* scores were recorded in children who were planned for orthodontic treatment prior to oral hygiene maintenance, after 2 weeks of orthodontic period, that is, during the wash out period, and during the run-in period of 2 weeks of milk product consumption (Table 2).

Salivary *S. mutans*

At the baseline, the maximum (10) number of subjects had the score of 2 followed by the score of 3 (7) and the scores of 0 and 1 (5 each). After 2 weeks of orthodontic treatment, the maximum (12) number of subjects had the score of 2, followed by the score 3 (11) and score 1 (3) (Table 2), which showed that during orthodontic treatment, there was an increase in the number of salivary S. mutans. Comparing the baseline values versus values after the intake of specified milk product in all the 3 groups, the maximum decrease in the S. mutans score was observed in subjects who consumed probiotic yoghurt, followed by the Indian curd and ultra-heated yoghurt groups (Table 3). Comparing the baseline versus the third reading (2 weeks after milk product consumption), only the probiotic yoghurt results were significant (p=0.014)*; whereas they were nonsignificant for the Indian curd (p=0.581) and ultra-heated yoghurt groups (p=1.000). It shows a significant effect of probiotic yoghurt to reduce the S. mutans count in the saliva, irrespective of low- or high-caries risk groups (Table 2).

When comparing the second (after 2 weeks of orthodontic treatment) and the third reading (2 weeks after milk product ingestion); it was found that probiotic yoghurt (Py) results were highly statistically significant (p=0.006)**; whereas they were nonsignificant in the Indian curd (p=0.068) and ultra-heated yoghurt groups (p=1.000). It shows a highly significant effect of probiotic yoghurt to reduce the S. mutans count in saliva of the high-caries risk group followed by Indian curd when compared with placebo ultra-heated yoghurt (Table 2, 3, 4).

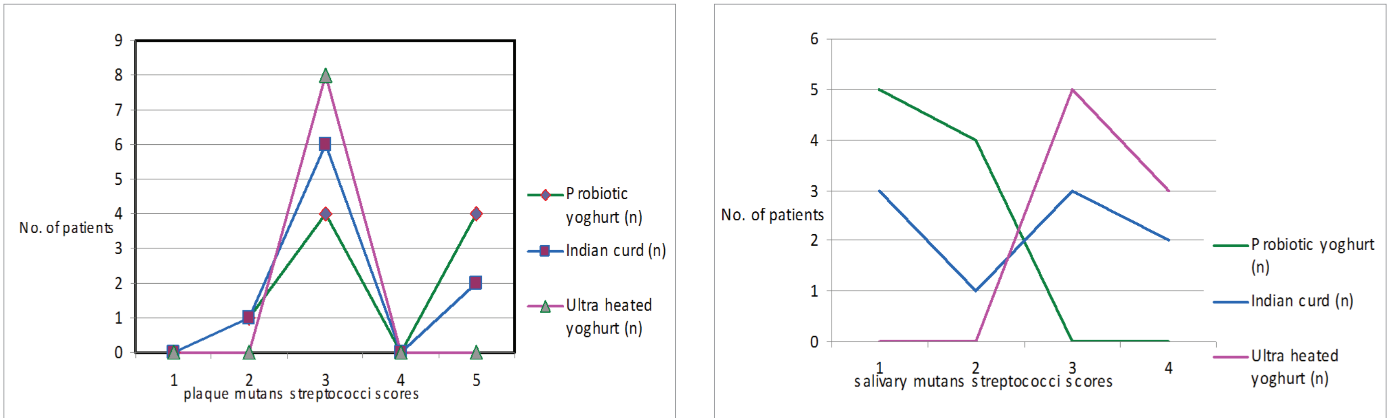

Figure 1. Graphic presentation of the salivary and plaque *Streptococccus mutans* count relation

Table 4. Wilcoxon-signed ranks test

Milk Product Group		2nd reading (Bp)-1st reading (Ap)	3rd reading (Cp)-1st reading (Ap)	3rd reading (Cp)- 2nd reading (Bp)
Indian curd	Z	-0.106a	-2.232a	-2.058a
	P value (2-tailed)	0.915	0.026*	0.040*
Probiotic yoghurt	Z	-1.604a	-2.636a	-2.049a
	P value (2-tailed)	0.109	0.008**	0.040*
Ultraheated yoghurt	Z	-1.414a	-1.000a	0.000b
	P value (2-tailed)	0.157	0.317	1.000

a: Based on positive ranks; b: The sum of negative ranks equals the sum of positive ranks; c: Wilcoxon signed-rank test

During orthodontic treatment, there was an increase in the number of banded tooth plaque associated salivary S. mutans. When comparing the baseline versus the third reading (2 weeks after milk product consumption), probiotic yoghurt showed a highly significant difference (p=0.008)** followed by Indian curd (p=0.026)* and ultra-heated yoghurt group (p=0.317). This shows a significant effect of probiotic yoghurt to reduce the plaque *S. mutans* count associated with banded teeth, irrespective of the low- or high-risk caries group (Figure 1).

Comparing of the second (after 2 weeks of orthodontic treatment) and third reading (after 2 weeks of milk product ingestion) indicated that both probiotic yoghurt and Indian curd groups showed a highly statistically significant difference (p=0.040)*, while the ultra-heated yoghurt group (p=1.00) was nonsignificant. This shows a significant effect of probiotic yoghurt in reducing the associated S. mutans banded tooth count in saliva of high-risk caries group followed by Indian curd when compared with placebo ultra-heated yoghurt (Table 2, 3, 4).

Correlation between the number of sugar exposures with salivary and plaque *S. mutans* scores

Out of 27 subjects, 3 subjects consumed snacks such as toffees, chocolates, Indian sweets (*Gajjak*) between the meals. It was observed that 2 subjects consumed toffees and chocolates once a week. One subject consumed toffees/Indian sweets once a day on all 7 days. The plaque and saliva samples for all these subjects were closely observed. Only one subject consumed sweets daily; even though the correlation between the salivary and plaque S. mutans counts 2 weeks of consuming of a specified milk product, and the number of sugar exposures was nonsignificant in our study.

DISCUSSION

All subjects showed an increase in the *S. mutans* count score after 2 weeks of orthodontic therapy, and half of them reached the highest scores both in the saliva and banded-tooth-associated plaque. This indicates that there is a rapid and definite increase in the caries risk in terms of the *S. mutans* count, even after 2 weeks of orthodontic therapy. Fixed orthodontic therapy should be considered as a definitive threat to oral health in terms of hard-tissue demineralization and caries because of an increased salivary S. mutans count (9). Milk products contain basic nutrients for the growing child; they are also safe for the teeth, with possible beneficial effects on the salivary microbial composition and inhibition of caries development due to their natural content of casein, calcium, and phosphorus (10). A dairy-based vehicle seems to be most promising because of its low cariogenic potential and a high buffer capacity (11). The archetypical probiotic food is yoghurt, and daily consumption of dairy products seems to be the most these are not consistent across species. The LAB abundance in curd increased rapidly at 12h of fermentation at room temperature and declined thereafter (10).

We used probiotic-containing milk products in the second part of our study in these orthodontic subjects with an increased caries risk. The effect of probiotic yoghurt consumption for 2 weeks on subjects undergoing fixed orthodontic therapy was found highly significant in terms of a decrease in the *S. mutans* scores. It shows that the ingestion of probiotic yoghurt can be a highly effective mean to decrease an increased caries risk in orthodontic subjects as far as the count of salivary *S. mutans* is concerned. Probiotic yoghurt shows a significant effect when used in these orthodontic subjects in terms of a decrease in the plaque *S. mutans* count. Reason behind the difference in the probiotic effectivity, that is, its better ability to reduce the salivary *S. mutans* count than the plaque *S. mutans* count, may be the localization of increased caries risk situation, as prophylaxis becomes highly compromised in these local areas adjacent to bands, and more aggressive and locally acting measures to reduce the *S. mutans* numbers will be required (12-14). In the plaque, a statistically significant difference was observed in probiotic yoghurt, when the baseline and third reading (after 2 weeks of milk product ingestion) was made. However, the comparison between the second and third reading revealed that both probiotic yoghurt and Indian curd were equally beneficial. Therefore, it can be concluded that probiotic yoghurt is an effective vehicle in decreasing the *S. mutans* numbers in orthodontic patients, but curd can also be considered beneficial in a developing country like India (15). It has been mentioned in the literature that the benefits of probiotics can be best exploited if started in early childhood, since the displacement of pathogenic bacteria by friendly bacteria can occur easily before a permanent establishment and colonization of microflora occurs (16, 17). The effect of probiotic is better in the oral cavity as it directly interacts with oral epithelium, thus reducing the incidence of caries, gingivitis, and malodor. While in the gut complex ecosystem exit; also, they depend on the colonization factors and resist the effect of peristalsis, which tends to flush out bacteria with food. Only those strains resistant to bile impart beneficial effects in the gut.

Observed changes in salivary microbiota provide evidence to clinicians for recommending probiotics to their patients in addition to "classical" oral hygiene practices and dietary counseling (18-20). Therefore, it can be concluded that the short-term consumption of probiotic-containing dairy products is highly effective in decreasing an increased *S. mutans* count in subjects undergoing fixed orthodontic therapy. Since, our study was a short-term study examining the effectivity of probiotics on the *S. mutans* level in subjects undergoing fixed orthodontic therapy, further long-term research is required to study beneficial effects of probiotics in groups with an increased risk.

CONCLUSION

- Probiotic yoghurt is significantly more effective in the reduction of *S. mutans* in the saliva than in the plaque in association with banded teeth of subjects undergoing orthodontic therapy.
- Indian curd decreases the *S. mutans* count in the plaque and saliva, but less effectively than probiotic yoghurt. Ultra-heated yoghurt does not impart any beneficial effects in reducing the *S. mutans* count in orthodontic patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Maharishi Markandeshwar deemed to be university**.**

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.G.; Design - S.G.; Supervision - S.G., M.S.; Data Collection and/or Processing - M.S., V.S.; Analysis and /or Interpretation - A.D.; Literature Search - N.J.; Writing Manuscript - M.S, S.G.; Critical Reviews - N.J., A.D.

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