Original Article

Effect of Probiotic Powdered Milk Containing *Lactobacillus paracasei* SD1 on The Level of Salivary Yeast in Orthodontic Treated Cleft Lip and Palate Patients

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Abstract

The objective of this study was to evaluate the effect of short-term consumption of probiotic powdered milk containing *Lactobacillus paracasei* SD1 on the level of salivary yeast in orthodontic treated cleft lip and palate patients. A total of 30 cleft lip and cleft palate patients who had been undergoing orthodontic treatment with fixed orthodontic appliances at the Dental Hospital, Faculty of Dentistry, Prince of Songkla University, were divided into two groups; the study and control groups. The study group, consisting of 15 subjects, received 10 g/50 ml/day of probiotic powdered milk containing *Lactobacillus paracasei* SD1, while the 15 subjects in the control group received 10 g/50 ml/day of normal powdered milk. Both groups were advised to drink the provided milk daily after breakfast for 30 days. The salivary yeast level, plaque index (PI) and salivary pH were investigated before milk consumption (I), immediately (T0) and at one week intervals after completed the 30-day period of milk consumption for 4 weeks (T1, T2, T3, T4). There was a statistically significant decrease of the salivary yeast level ($p < .05$) found at T0-T4 within the study group comparing to the baseline. However, there was no statistically significant change within the control group. When compared between the two groups, there was a statistically significant difference in the percent change from baseline level of salivary yeast at T0-T4. No statistically significant difference was found in the salivary pH and plaque index between the two groups throughout the study. In conclusion, the short-term consumption of probiotic powdered milk containing *Lactobacillus paracasei* SD1 could decrease the salivary yeast counts in the orthodontic treated cleft patients.

Key words: Clefts; *Lactobacillus paracasei* SD1; Probiotic; Yeast

Introduction

Surgical repair of the lip and palate tend to result in poor skeletal, especially in transverse and antero-posterior planes of maxilla, and dental growth of the clefts. To develop the arches and align the teeth, several phases of orthodontic treatment are unavoidable. Patients who undergoing orthodontic therapy, whether with fixed or removable appliance, have oral ecologic changes such as a low pH environment, increased retentive sites for microorganisms, and increased retention of food particles, which may lead to increased proportions and absolute numbers of oral microflora.[1-3]

Yeast, a normal inhabitant of the oral cavity, is the most common cause of oral fungal infections. Age, genetic, hormonal, iatrogenic, systemic and local factors predispose clinical manifestations of the disease.[4] From Ahluwalia et al.[5] and Rawashd et al.,[6] yeast played an important role as one of microbiota infection in oral cavity of the clefts.

Probiotic bacteria are known to benefit the host by inhibiting the growth of pathogenic microorganisms. The most common probiotic strains proposed for oral cavity belong to the genera *Lactobacillus* and Bifidobacterium.[7] The bacterial strains used in various studies have been isolated from different sites of the human body such as *L. reuteri* ATCC 55730[8] and *Bifidobacterium animalis* subsp. *lactis* DN-173010 from human intestine[9] or the lactobacilli species, *L. fermentum, L. rhamnosus, L. salivarius, L. casei, L. acidophilus* and *L. plantarum*, were recovered from saliva.[10] Great variation in the inhibitory effects among the strains of the same species was shown.[11] Among the *Lactobacillus* strains that showed a strong inhibitory effect in the study of Teanpaisan et al.,[12] *L. paracasei* SD1 and *L. fermentum* SD6 were the most efficient among the tested strains of individual species.

Previous studies have suggested that probiotic supplements in dairy products may affect the oral microbial ecology.[8,9,13-15] However, the possible effect of *Lactobacillus paracasei* SD1 on the salivary yeast level in orthodontic treated cleft patients has not previously been reported. The aim of the study was to investigate the effect of short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 on the salivary yeast level in orthodontic treated cleft patients

Materials and methods

Participants:

The study was approved by the Ethics Committee of the Faculty of Dentistry, Prince of Songkla University. Subjects and/or their parents gave informed consent to participate in the study. Thirty cleft lip and palate patients from the Orthodontic clinic, Dental hospital, Faculty of Dentistry, Prince of Songkla University were recruited in this study based on the following inclusion criteria:

1. Undergoing treatment with fixed orthodontic appliances for at least 3 months with bracket attachments on at least 20 permanent teeth.
2. No systemic disease and without orofacial clefting as part of a craniofacial syndrome
3. No use of systemic antibiotics, antimicrobial drugs or any probiotic products within the past 2 weeks
4. No history of milk allergy and/or lactose intolerance
5. No active untreated carious lesions and no candida infection

Study Protocol

The prospective investigation was a double blinded randomized placebo controlled study design. The subjects were divided into two groups, the study and control group, by simple randomized allocation. There were 15 subjects in the study group and 15 subjects in the control group. The study group received probiotic milk and the control group received normal powdered milk. All subjects were advised to take the received milk daily after breakfast for 4 weeks. No tooth brushing was allowed for at least 1 hour after milk consumption. During the experimental period, all subjects were instructed not to receive any other form of probiotic products and to maintain their normal oral hygiene habits throughout the study.

Probiotic and control milks

The powdered milk used in this study was manufactured by the Faculty of Agro-Industry, Prince of Songkla University, and divided into two forms of 10 g. powdered milk per pack. The probiotic milk contained *Lactobacillus paracasei* SD1 at a concentration within the recommended range of viable counts of $7.5\pm0.20 \times 10^8$ CFU g^{-1}, while the normal milk was without viable bacteria. A pack of powdered milk mixed in 50 ml. water was instructed for daily intake after the breakfast time for 30 days. All participants were recommended to keep the products in the refrigerator when they were not in use.

Data records and collections

Demographic data was recorded by questionnaire and side effects of milk consumption were recorded by interviewing. Oral clinical examinations (plaque index,[16] and salivary pH), salivary collection and culture were recorded at initial (I), immediately after completed milk consumption (T0) and one week interval after completed milk consumption for 4 weeks (T1-T4). Salivary pH was tested by dipping the pH-indicator strip (Merck universal indicator strips) in un-stimulated saliva of each subject for 10 seconds; the changing color was compared with the standard chart.

Yeast evaluation was performed using an oral rinse method.[17] After holding ten millimeters of sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.0) in the mouth for one minute, the rinse was collected into a sterile container. Each rinse was centrifuged (4,000 rpm; 10 min), the supernatant was removed, and the pellet was re-suspended in 1 ml of PBS. The mixture was diluted with sterile phosphate-buffered saline by serial dilution into 1:10, 1:100, 1:1000, 1:10,000 dilutions. A portion (10 µl) of each dilution was spread on Sabouraud Dextrose Agar and incubated in an aerobic chamber at 37 C for 48 hours. The yeast colonies were identified on the basis of their morphology, counted under a microscope with 10 times magnification and multiplied by the dilution factor to yield the CFU/ml of the original oral rinse sample and converted to $\log_{10}$ value.

The summary of all procedures are demonstrated in Diagram 1.

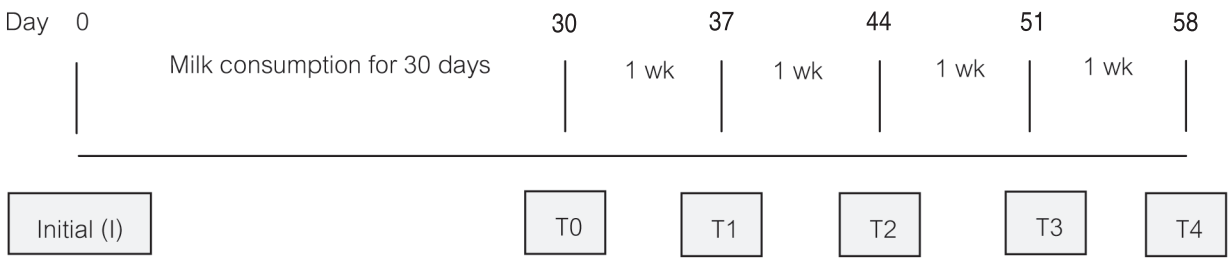

Diagram1: Clinical procedure

Statistical analysis

All numerical data are presented as means and standard deviations. The numbers of colony count of yeasts were presented as $\log_{10}$ CFU/ml and percent change from baseline were analyzed using Wilcoxon Signed-Rank test and Mann-Whitney U-test. A p-value of less than 0.05 was considered to be statistically significant.

Results

Descriptive information of the 30 cleft lip and cleft palate patients was shown in Table 1. All subjects completed the trial without any side effects such as diarrhea or allergies were reported. At baseline, there was no statistically significant difference between the groups concerning the distribution of salivary pH and plaque index but a statistically significant difference in the salivary yeast counts between the groups. (Table 2)

Table 1 *Descriptive information of the subjects*

Group	Number of subjects			Mean ages (years±s.d.)
	Male (%)	Female (%)	Total (%)	
Probiotic	7 (46.7)	8 (53.3)	15(100)	19.0±4.24
Control	4 (26.7)	11 (73.3)	15(100)	19.4±3.11
Total	11 (36.7)	17 (63.3)	30(100)	19.2±3.66

Fig. 1 *Diagram of the selection of study samples and data collection*

Table 2 *The salivary yeast counts, salivary pH and plaque index at base line*

Parameters	Control group	Study group	*p*-value
	Mean scores	Mean scores	
Yeast[a]	3.14±1.43	4.33±1.04	0.018*
Salivary pH	6.58±0.46	6.51±0.43	0.859
PI	2.96±0.38	3.04±0.47	0.885

[a] The value in the table denotes the log10 CFU/ml.

* $p < .05$(Mann-Whitney U-test)

Table 3 *Distribution of salivary yeast scores at pre and post-milk consumption (within groups)*

Groups	Yeast[a]	*p*-value
	Mean scores	
Control group		
Initial	3.14±1.43	
T0	3.05±1.40	0.528
T1	3.37±1.72	0.402
T2	3.35±1.39	0.306
T3	3.79±0.89	0.208
T4	3.63±1.67	0.052
Study group		
Initial	4.34±1.05	
T0	3.95±0.95	0.009*
T1	3.82±1.08	0.001*
T2	3.88±1.12	0.013*
T3	4.02±0.94	0.008*
T4	3.93±0.94	0.008*

[a] The value in the table denotes the log10 CFU/ml.

* $p < .05$(Wilcoxon Signed-Rank test)

In the study group, the salivary yeast counts at the immediate and all periods after milk consumption (T0-T4) decreased significantly ($p < .05$) when compared with the initial record. However, there was no statistically significant change in the control group. (Table 3)

When comparing between the groups, significantly higher yeast counts at baseline (I) was found in the study group. So, the percent change of yeast counts from baseline at T0-T4 were used to compare between the two groups, the statistically significant differences ($p < .05$) of percent changes of salivary yeast count were found at T0-T4 between the study and control group. (Table 4)

Table 4 *Distribution of the salivary yeast counts after milk consumption (between groups)*

Time	Control group[a]	Study group[a]	*p*-value
	% change from baseline	% change from baseline	
T0	-1.65 ±11.41	-8.47±10.16	0.048*
T1	0.88±11.72	-10.72±9.47	0.031*
T2	4.51±21.21	-9.46±10.19	0.035*
T3	7.62±21.54	-10.79±10.38	0.018*
T4	10.17±16.50	-8.60±10.19	0.002*

[a] The value in the table denotes the log10 CFU/ml.

* $p < .05$(Mann-Whitney U-test)

Table 5 *Comparison of salivary pH value between group before and after milk consumption*

Time	Control group	Study group	*p*-value
	Mean scores	Mean scores	
Initial	6.58±0.46	6.51±0.43	0.859
T0	6.62±0.45	6.56±0.42	0.650
T1	6.61±0.44	6.59±0.42	0.925
T2	6.66±0.43	6.62±0.46	0.948
T3	6.63±0.42	6.64±0.42	0.752
T4	6.62±0.40	6.60±0.47	0.857

* $p < .05$(Mann-Whitney U-test)

There were no statistically significant changes in salivary pH or plaque index within the groups or between the groups throughout the study. (Table 5 and 6)

Table 6 *Comparison of plaque index scores between groups before and after milk consumption*

Time	Control group	Study group	*p*-value
	Mean scores	Mean scores	
Initial	2.96±0.38	3.04±0.47	0.885
T0	2.92±0.35	3.02±0.47	0.724
T1	2.79±0.39	3.03±0.46	0.301
T2	2.86±0.38	3.14±0.49	0.198
T3	2.91±0.38	3.01±0.50	0.918
T4	2.92±0.32	3.03±0.44	0.663

* $p < .05$(Mann-Whitney U-test)

Discussion

Probiotics have been proven to be advantageous in preventing various diseases.[18] It is believed that at least some probiotic mechanisms may also play a role in the oral cavity.[19] Although many studies with *L. rhamnosous* GG, *L. reuteri* have defined their potential in interacting with *Streptococcus mutans* by reducing the number of this cariogenic pathogen, thus suggesting a role of probiotics in caries prophylaxis.[15,19] The potential beneficial effects of probiotics containing these microorganisms on salivary yeast counts have only poorly been studied. *Lactobacillus paracasei* SD1[12] is a normal oral microorganism which was isolated from the mouths of caries-free children and was chosen to be the probiotic supplement in this study because of its convenience of storage and use, long shelf life, health benefits, and low cariogenic potential.

In this study, the effect of short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 in cleft patients who had specific oral environment was investigated. The intervention was started 3 months after the orthodontic fixed appliances were placed to avoid the confounding effect of an immediate decrease in the salivary yeast counts that may take place at appliance insertion.[20]

Ahluwalia et al.[5] found from their study in children aged 6–16 years, with (n = 81) or without (n = 61) cleft palates, that the salivary levels of yeasts were significantly greater in the cleft palate children than in the control group (1.70±0.17 and 0.69±0.16 $\log_{10}$ CFU/ml, respectively). Rawashd et al.[6] found that both cleft and healthy control group showed high prevalence rates of yeasts (67.3% and 20.5%, respectively) with the difference between patients with cleft and controls being statistically significant. In Thailand, Teanpaisan and Nittayanata[21] found the range of 1.90-4.54 $\log_{10}$ CFU/ml yeast count in healthy Thai subjects. At baseline in this study, we also found that our subjects showed a high level of salivary yeast counts, 3.14±1.43 and 4.33±1.04 $\log_{10}$ CFU/ml, respectively.

Hatakka et al.[22] found that probiotic cheese containing *L. rhamnosus* GG (ATCC 53103), *L. rhamnosus* LC705 and

Propionibacterium freudenreichii ssp *shermanii* JS reduced the risk of high yeast counts and could be effective in controlling oral Candida and hyposalivation in the elderly. Ahola et al.[23] found that probiotic cheese containing *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC705 tended to reduce the level of salivary yeasts in healthy adults. Our study also found that the probiotic milk containing *Lactobacillus paracasei* SD1 significantly decreased the salivary yeast counts after completed milk consumption at least 4 weeks in the study group while no statistically significant difference was found in the control group.

There were no statistically significant changes among the study groups in salivary pH and plaque index during all the after completed milk consumption study periods. This result was reinforced in the in vitro study that this strain produces less acid than other strains.[12] This might imply that the powdered milk containing *Lactobacillus paracasei* SD1 had no apparent adverse effect on salivary pH and plaque index.

Regarding further study, it would be interesting to study more clearly whether the stable ecology was affected by this intervention or not and determine the long-term effect of probiotic bacteria on the oral microbial counts with a larger sample size.

Conclusion

The study demonstrated that short-term consumption of powdered milk containing *Lactobacillus paracasei* SD1 could reduce the salivary yeast counts in orthodontic treated cleft patients. No adverse effect on salivary pH and plaque index was evident. It is thus concluded that *Lactobacillus paracasei* SD1 could be a promising probiotic strain for specific oral ecology, but the clinical application needs further study.

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บทวิทยาการ
Original Article

ผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อปริมาณยีสต์ในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการรักษาทางทันตกรรมจัดฟัน

ชลธิรา แซ่ตั้ง
ทันตแพทย์
กลุ่มงานทันตกรรม โรงพยาบาลหนองแค สระบุรี
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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันต่อปริมาณยีสต์ในน้ำลายของผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการรักษาทางทันตกรรมจัดฟันชนิดติดแน่น โดยทำการทดลองจากกลุ่มตัวอย่างผู้ป่วยปากแหว่งเพดานโหว่ที่กำลังอยู่ระหว่างการรักษาทางทันตกรรมจัดฟันโดยการใช้เครื่องมือจัดฟันชนิดติดแน่นที่โรงพยาบาลทันตกรรม คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ จำนวน 30 รายถูกแบ่งเป็น 2 กลุ่มคือกลุ่มศึกษาและกลุ่มควบคุม โดยกลุ่มศึกษาประกอบด้วยผู้ป่วยจำนวน 15 ราย ซึ่งได้รับนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันปริมาณ 10 กรัม/น้ำ 50 มล./วัน ในขณะที่กลุ่มควบคุมประกอบด้วยผู้ป่วยจำนวน 15 ราย ได้รับนมผงปกติปริมาณ 10 กรัม/น้ำ 50 มล./วัน ทั้งสองกลุ่มได้รับคำแนะนำให้ดื่มนมในช่วงหลังรับประทานอาหารเช้าทุกวัน เป็นระยะเวลา 30 วัน ปริมาณยีสต์ ดัชนีแผ่นคราบจุลินทรีย์และความเป็นกรดด่างของน้ำลายจะถูกวิเคราะห์ที่ระยะเวลาก่อนรับประทานนม (I) หลังรับประทานนมครบ 30 วัน (T0) และหลังระยะเวลาที่รับประทานนมครบต่อไปอีกสัปดาห์ละ 1 ครั้ง เป็นเวลา 4 สัปดาห์ (T1,T2,T3,T4) ผลการศึกษาพบว่า ในกลุ่มศึกษามีปริมาณยีสต์ในน้ำลายลดลงอย่างมีนัยสำคัญทางสถิติ ($p < .05$) ในทุกช่วงเวลาหลังรับประทานนม (T0-T4) จากปริมาณเชื้อตั้งต้น แต่ไม่พบความแตกต่างกันทางสถิติ ในกลุ่มควบคุม เมื่อเปรียบเทียบระหว่าง 2 กลุ่มพบว่าเปอร์เซ็นต์การเปลี่ยนแปลงปริมาณยีสต์ในน้ำลายในกลุ่มศึกษาลดลงอย่างมีนัยสำคัญทางสถิติ ในทุกช่วงเวลาหลังรับประทานนม (T0-T4) เมื่อเปรียบเทียบกับกลุ่มควบคุม และไม่พบความแตกต่างทางสถิติของค่าความเป็นกรดด่างของน้ำลาย ค่าดัชนีแผ่นคราบจุลินทรีย์ตลอดการศึกษาในทั้งสองกลุ่ม สรุปได้ว่าในระยะสั้นการรับประทานนมผงโพรไบโอติกแลคโตบาซิลลัสพาราเคซิอายเอสดีวันมีผลในการลดปริมาณยีสต์อย่างมีนัยสำคัญทางสถิติในผู้ป่วยปากแหว่งเพดานโหว่ที่ได้รับการรักษาทางทันตกรรมจัดฟัน