Mutans streptococci estimation in saliva before and after consumption of probiotic curd among school children

Manish Bhalla, Navin Anand Ingle, Navpreet Kaur, Pramod Yadav

Department of Public Health Dentistry, Kanti Devi Dental College and Hospital, Mathura, Uttar Pradesh, India

Corresponding author (email: <drmanishbhalla@hotmail.com>)
Dr. Manish Bhalla, Department of Public Health Dentistry, Kanti Devi Dental College and Hospital, Mathura, Uttar Pradesh, India.

Abstract

Background: According to the World Health Organization, probiotics are live microorganisms which when administered in adequate amounts and confer a health benefit to the host. Use of probiotics in children to improve oral health may lead to non-pathogenic bacteria replacing cariogenic bacteria. Dairy foods like cheese, curd, and milk are considered useful vehicles for probiotic bacteria. Aim: To compare the levels of salivary mutans streptococci before and after consumption of probiotic curd. Materials and Methods: Thirty school children of Mathura city who were caries free, in the age group of 12–14 years, were selected and divided equally into group I and II which were given 200 ml probiotic curd and normal curd for 7 days, respectively. Assessment of saliva samples was done at baseline, 1 h after consumption, and on the 7th day. Mitis Salivarius Bacitracin Agar was used for analysis. The number of colonies was counted and subjected to statistical analysis using unpaired Student’s t-test. Results: The study showed mark reduction in salivary mutans streptococci counts in saliva after 1 h and on the 7th day in the probiotic group. Results were found to be statistically significant (\( P \leq 0.05 \)) when the differences in the reduction of mutans streptococci counts with probiotic curd and normal curd at 1 h and on the 7th day were compared. Conclusion: Pathogenic microorganisms could be displaced by probiotic bacteria. Thus, use of probiotic products could be exploited for the prevention of enamel demineralization.

Key words: Curd, mutans streptococci, probiotic

INTRODUCTION

Elie Metchnikoff, a Ukrainian born Nobel Prize laureate, observed the positive beneficial effect of some bacteria on human health and suggested that these beneficial bacteria can be used to replace harmful microbes in the body. Since 20th century, different microorganisms have been used for their ability to prevent and cure diseases, while the commonly prescribed antibiotics are rendered useless by antibiotic resistance. Then in 1994, the World Health Organization (WHO) deemed probiotics to be the next most important immune defense system. Lilly and Stillwell first coined the term probiotic which means “for life.” Probiotics are defined as “live microorganisms which when administered in adequate amounts, confer a health benefit on the host.” Most commonly found probiotic strains are Lactobacillus and Bifidobacterium species. Bacterial strains that have been tested for probiotic action in the oral cavity include lactobacilli species (Lactobacillus acidophilus, Lactobacillus rhamnosus GG, Lactobacillus johnsonii, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus paracasei \(^{11}\)), Bifidobacterium species (Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium animalis strain DN-173 010), and others (Streptococcus salivarius, Weissella cibaria).\(^{11}\)
The possible impact of probiotics on the oral health is less explored. Dairy foods such as cheese, yoghurt, and milk are considered useful vehicles for probiotic bacteria, but an ideal administration vehicle has yet to be identified. Ideally, the delivery should be suitable for all ages, especially for very young children since it has been suggested that exposure early in life may facilitate a permanent installation of health-promoting strains. In this context, ice-cream and curd are interesting probiotic foods, as they are popular and universally liked.[2]

As a profession, we are slowly moving away from the purely surgical approach to treating dental caries. Science is providing us the tools to diagnose and treat the infection before it causes irreversible damage. Replacement therapy in the form of probiotics is an alternate and promising way to combat infections by using harmless bacteria to displace pathogenic microorganisms. Probiotics have been used successfully for treating gastrointestinal disorders in the medical field. The use of probiotics in dentistry has shown to reduce the cariogenic biofilm in various studies. Probiotic strains may help in preventing new caries in already treated population.[3] The various means by which probiotics can be administered for oral health purpose are: cheese, yoghurt, lozenges, tablets, mouth rinse, capsule.[1]

MATERIALS AND METHODS

In this study, the levels of salivary mutans streptococci were compared before and after consumption of probiotic curd.

Thirty children were randomly selected from S. R. Public School Shiksha Samiti, Mathura for the study. The children were selected and equally divided into group I (probiotic curd group) and group II (plain curd group) comprising 15 children in each group, who were given 200 g probiotic curd (which contained B. lactis 12) and plain curd, respectively, for 7 days. Curd was given once daily and the subjects were instructed to refrain from subjects were given probiotic curd to consume once daily and were instructed to refrain curd consumption any other time. The subjects were, however, encouraged to maintain their normal oral hygiene habits. No tooth brushing was allowed for at least 1 h after eating the curd.

Inclusion criteria

- Age group of the children was 12–14 years
- All permanent teeth should have been erupted (except 3rd molars)
- No clinically detectable caries
- No history of any preventive dental treatment
- Children must be present on the day of examination
- Informed consent from the parent/guardian was obtained.

Exclusion criteria

- Severely ill children
- Medically compromised children
- Children who had been on medication in the last 6 months
- Children undergoing orthodontic treatment.

Ethical clearance was obtained prior to the start of the study from the ethical committee of K. D. Dental College, Mathura.

Collection of saliva was done after the clinical examination. Children were made comfortable and asked to swallow preexisting saliva in order to clear the mouth of any residual saliva. Sterile hard plastic container was given to each student and the student was asked to split the saliva into it. All the saliva samples of participants were decoded during the period of sample collection and processing. The samples collected were handed over to the Rangeshwar pathology laboratory, Mathura city for analysis on the same day. The samples were precoded and not disclosed to the technician. In the laboratory, samples were stored at room temperature (17°C–25°C) prior to the analysis. Assessment of saliva was done at baseline, at 1 h, and on the 7th day by using Mitis Salivarius Bacitracin Agar. Sample was inoculated on the Mitis Salivarius Bacitracin Agar. The plates were incubated at 37°C anaerobically. Colony characteristics were studied after 72 h. Streptococcus mutans in saliva was determined by using a colony counter and the number of colony forming units was counted.[4]

Statistical analyses

The statistical analysis was performed using SPSS version 16 (IBM Corporation). was used for statistical analysis. The mean and standard deviation for S. mutans count in samples were determined using unpaired Student’s t-test.

RESULTS

In this study, mean salivary mutans streptococci count at baseline for probiotic curd and plain curd groups was $211.6 \pm 24.5$ and $215.8 \pm 22.1$, respectively.
respectively. Mean salivary mutans streptococci count at 1 h after consumption of probiotic curd and plain curd was 185 ± 12.5 and 211.3 ± 21.6, respectively. When compared after 7 days, mean salivary mutans streptococci count after consumption of probiotic curd and plain curd was 150.6 ± 8.11 and 213.3 ± 25.6, respectively. When comparison of the mean salivary mutans streptococci was done 1 h and 7 days after consumption of probiotic and plain curd, the results were found to be statistically significant (P ≤ 0.05) [Table 1].

DISCUSSION

Bacteriotherapy is a novel and promising concept for combating infections and preventing dental caries. Studies on probiotics were performed to validate the survival and positive effects of probiotic bacteria *Bifidobacterium lactis* Bb-12 within the human body, including immune response and gastrointestinal health in young children, but studies concerning probiotics and dental health are limited.[5]

Since the late 1980s, a range of dairy products containing bifidobacteria have been marketed in a number of countries worldwide, and studies have been performed to validate the survival and positive effects of *Bifidobacterium*. Pathogenic microorganisms could be displaced by probiotic bacteria. Thus, use of probiotic products could be exploited for the prevention of enamel demineralization. Probiotic technology represents a breakthrough approach to maintain oral health by utilizing the natural beneficial bacteria commonly found in healthy mouth to provide natural defense against those bacteria thought to be harmful to teeth and gums.[7] Studies carried out to validate the beneficial effect of probiotic curd on the oral ecology are few. Therefore, our study was done to estimate the effect of probiotic curd on salivary mutans streptococci. The product used for the study was well accepted by the participants, which was expected since curd eating is a life-long tradition followed in India.

Use of probiotics may reduce the cost of conventional therapy and prevention programs for the management of oral disease. It is an attractive idea of replacing harmful microorganisms with non-harmful, inactivated, or genetically modified bacteria. With the focus on disease prevention and optimal health for all ages, the potential for probiotics’ use is enormous. Awareness should be created about the aspect of oral disease therapy and encourage the implementation of the concept of “food rather than medicine.” The existence of probiotics in the indigenous oral microflora of humans should be explored because these bacteria offer the advantage of being perfectly adapted to the oral ecosystem. For better understanding of probiotics,

| Time interval | Curr | Mean | Standard deviation | P  |
|---------------|------|------|--------------------|----|
| Baseline      | Probiotic | 211.6| 24.5               | 0.62|
|               | Plain    | 215.8| 22.1               |     |
| 1 h           | Probiotic | 185.0| 12.5               | 0.0002*|
|               | Plain    | 211.3| 21.6               |     |
| 7 days        | Probiotic | 150.6| 8.11               | 0.0001*|
|               | Plain    | 213.3| 25.6               |     |

*P≤0.05 = Statistically significant
scientific research is needed to broaden their potential applications.

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**REFERENCES**

1. Vishnu HP. Probiotics and oral health. In: Virdi M, editor. Oral Health Care: Pediatric Research, Epidemiology and Clinical Practices. Bangalore, India: Vydehi Institute of Dental Sciences and Research Centre; 2012. p. 195-204.
2. Chinnappa A, Konde H, Konde S, Raj S, Beena JP. Probiotics for future caries control: A short-term clinical study. Indian J Dent Res 2013;24:547-9.
3. Ranganathan J, Vaidy R. Preventing dental caries the probiotic approach. The Journal of Ahmedabad Dental College and Hospital 2012;2:60-3.
4. Wan AK, Seow WK, Walsh IJ, Bird PS. Comparison of five selective media for the growth and enumeration of Streptococcus mutans. Aust Dent J 2002;47:21-6.
5. Hasslöf P, Hedberg M, Twetman S, Stecksén-Blicks C. Growth inhibition of oral mutants streptococci and candida by commercial probiotic lactobacilli—an in vitro study. BMC Oral Health 2010;10:18.
6. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutants streptococci and lactobacilli in young adults. Acta Odontol Scand 2005;63:317-20.
7. Dhawan R, Dhawan S. Role of probiotics on oral health: A randomized, double-blind, placebo-controlled study. J Interdiscip Dent 2013;3:71-8.
8. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutants streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod 2009;31:407-11.
9. Zhu Y, Xiao L, Shen D, Hao Y. Competition between yogurt probiotics and periodontal pathogens in vitro. Acta Odontol Scand 2010;68:261-8.
10. Singh RP, Damle SG, Chawla A. Salivary mutants streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing Bifidobacterium lactis Bb12 and Lactobacillus acidophilus La5. Acta Odontol Scand 2011;69:389-94.
11. Jindal G, Pandey RK, Agarwal J, Singh M. A comparative evaluation of probiotics on salivary mutants streptococci counts in Indian children. Eur Arch Paediatr Dent 2011;12:211-5.
12. Chung LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic lactobacillus paracasei effect on cariogenic bacterial flora. Clin Oral Investig 2011;15:471-6.

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