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Effect of Commercially Available Probiotic Ice-Cream On Salivary Levels of Streptococcous Mutans, Lactobacillus and Candida Albicans

[1] Dr Vivek S, [2] Dr Shwetha R
Senior lecturer, MDS in Oral and Maxillofacial Surgery, The Oxford Dental College and Hospital Bengaluru, India.
Senior lecturer, MDS in Public Health Dentistry, The Oxford Dental College and Hospital Bengaluru, India

Abstract:
Background: *Probiotic bacteria are live microbial food supplements that beneficially affect the host by improving its intestinal balance. Non – pathogenic microorganism, such as strains of Lactobacilli or Bifidobacteria, can occupy a space in human biofilm that otherwise would be colonized by pathogens and are thought to reduce the risk of some infectious disease. The aim of the present study was to examine whether consumption of commercially available ice-cream containing probiotics can affect the levels of salivary Streptococcus mutans, Lactobacillus and Candida albicans in young adults.*
Methodology: *Randomized control trail with parallel arm was designed, 20 healthy young adults (21-23years) were assigned to two equal sized probiotic group and control group and were followed over a period of 10 days after the consumption of allotted ice cream. The Colony Forming Units (CFU) of the study organisms was counted at baseline and immediately after intervention. Mean and standard deviations of the colony forming units count were tabulated and Wilcoxon Signed Rank Test was used for intra group comparison of colony and Mann-Whitney U-test was used for inter group comparison of the colony counts at baseline and after intervention.*
Results: *A significant reduction of salivary streptococcus mutans (p value=0.00) was achieved after consumption of probiotic ice cream. The salivary lactobacillus count was increased among probiotic group. There was reduction in Candida albicans count among probiotic group post intervention (p=0.035).*
Conclusion*: Results showed that potential consumption of probiotic ice cream may reduce the saliva levels streptococcus mutans and Candida albicans.*
Keywords: *probiotic ice cream, Candida albicans, lactobacillus, streptococcus mutans, saliva levels.*

I. Introduction

Probiotics, which means, "for life," have been used for centuries as natural components in health-promoting foods. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The most commonly used strains belong to the genera Lactobacillus and Bifidobacterium, genera that are commonly found in the oral cavity, including caries lesions. [1]

Mechanisms of action explaining beneficial probiotic effects include modulation of host immune response leading to strengthening of the resistance to pathogenic challenge, alteration of the composition and metabolic activity of host microbiota at the specific location, enhancement of the non-specific and specific immune response of the host; production of antimicrobial substances and competition with pathogens for binding sites. The potential health benefits that could be provided by probiotics in non-intestinal applications have to date received little attention. Considering the particular activities of probiotics and their inhibitory effect on the growth of pathogens, research interest has been extended to the oral cavity where probiotics may also exert their therapeutic or preventive effect on the development and progression of common oral diseases mainly dental caries, periodontal disease and oral candidiasis.[2]

Probiotic food constitutes a sizeable part of the functional food market, and continues to grow at an exponential rate in parallel with growing consumer awareness of the role of diet in health maintenance. After many years of popularity in the Japanese and European markets, manufacturers of these products are venturing into new markets, including the Arabian Gulf region and India as evidenced by the variety of probiotic food products now available in supermarkets and health food stores. However, this commercial exploitation of the probiotic food concept is still associated with a large body of unsubstantiated claims[3, 4].

The development of dairy products containing probiotic bacteria (bifidobacteria and intestinal lactobacilli) such as yoghurt, fermented milk products and cheese, cultured buttermilk, low-acidity yogurts, ice

cream, and powder preparations which is currently, an extremely important topic with industrial and commercial consequences. The best way of administration has yet to be identified.[5]

Ideally the delivery should be suitable for all age especially for young children; since it has been suggested that exposure early in life may facilitate a permanent installation of health promoting strains. In this contest the incorporation of probiotic bacteria into ice-creams is highly advantageous since, in addition to being a rich food from the nutritional point of view, containing dairy raw material, vitamins and minerals in its composition, it is usually consumed by everybody, being well accepted by the public.[1] Therefore, the search for effective, caries-preventing probiotic microorganisms and vehicle for delivery appears to be a promising research avenue. The aim of the present study was therefore to determine and compare the effect of short term consumption of commercially available probiotic and nonprobiotic ice-creams on salivary levels of Streptococcus mutans, Lactobacillus and Candida albicans.

II. Materials And Methods

Study group

The study group comprised twenty two healthy young adults aged 21-22 years who after receiving verbal and written information, volunteered to participate in the study. Subject with a history of systemic antibiotic or topical fluoride treatment within the 6 week prior to start of the study were not included. The individuals who habitually consumed probiotic or xylitol chewing gums were also excluded from the study. All subjects had good oral health with no open or untreated caries lesions, no signs of either gingivitis or periodontal disease, and no known allergy to ingredients in the study products. All the study subjects claimed daily tooth brushing habits using fluoridated toothpaste. During the experimental period, two participants were excluded from the study due to systemic antibiotics usage and violation of study protocol. Thus, the final results were based on 20 young adults.

Study design

The study was a triple blinded, randomized control clinical trial with parallel design with duration of 10 days. All the twenty two study subjects after obtaining consent were randomly allocated to one of two equally sized probiotic group and control group. Subjects were instructed to eat a cup (125ml) of allotted ice cream on a single occasion at noon with meals, daily for a period of 10 days. The subjects were encouraged to maintain their normal oral hygiene habits for 10 day period.

Ethical guidance: The study protocol was approved by the ethical committee of College of Dental Sciences, Davangere, Karnataka.

Intervention

The probiotic ice-cream, Amul prolife probiotic wellness® contained BB12 (Bifidobacterium bifidum) and LA-05 (Lactobacillus acidophilus) strains (as per details received from the company), while the control Amul ice-cream®, contained the same ingredients as the probiotic ice except for the presence of viable bacteria. The probiotic and the control ice creams were similar in terms of flavor (vanilla chocolate), taste and consistency. The test ice creams were delivered in white cups marked "A" and "B". The content was unknown to the test subjects and to the clinician responsible for the samplings. The code was not unveiled until after the statistical calculations.

Microbial evaluation

Paraffin-stimulated whole saliva were collected immediately before (baseline) and after 10^{th} day of intervention (follow-up). After a thorough rinse in water, the saliva was collected, over the course of 5mins, directly into a graded test tube and salivary flow rate was measured. Hundred micro liters of saliva was diluted with 1 ml of sterile peptone water to obtain 1:10 dilution of saliva. 100 µl of the diluted saliva was further added to 1 ml. of sterile peptone water to obtain a dilution of 1:100. This procedure was repeated again to obtain a dilution of 1:1000. This dilution of saliva was used for microbial analysis.

Salivary counts of Streptococcus mutans were quantified in the laboratory by culturing on Mitis Salivarius Bacitracin (MSB) agar, Lactobacillus on Rogosa agar and Candida albicans on Sabouraud's dextrose agar. The MSB agar plates were incubated for 48 hours at 37^{o} C, anaerobically using candle jar. The Rogosa SL agar plates were incubated for 48 hours at 37^{o}C, aerobically in the incubator. The Colony Forming Units (CFU) of each organism, were counted based on their morphology on the culture plates using the Colony Forming Units Counter.

Statistical method

The data were processed with statistical package for social sciences (SPSS) Version 17 software. Mean and standard deviations of the colony forming units count were tabulated and Wilcoxon Signed Rank Test was used for intra group comparison of colony counts at baseline and after intervention among the groups. Mann-Whitney U-test was used for inter group comparison of the colony counts at baseline and after intervention. p-value <0.05 was considered statistically significant.

III. Results

All the subjects had a stimulated saliva secretion rate within normal limits (1.0-2.5ml/min).

There were no difference between probiotic and non probiotic group at baseline concerning the microbial levels (table I). A statistically significant reduction of salivary streptococcus mutans was registered after ten day consumption of probiotic ice-cream among probiotic group(case) as compared with baseline with p value=0.005 (table2) there was also statistically significant reduction of salivary streptococcus mutans among probiotic group as compared to nonprobiotic group(control) with p value=0.00 (table1). There was no significant difference in the mutans count among nonprobiotic group after intake of control ice- cream as compared to baseline (table II).

There was no statistically significant changes appeared between the baseline and follow up samples in salivary lactobacilli counts between two groups (table I). As compared to baseline there was significant increase in lactobacillus count among the probiotic group after intervention (p value=0.017). There was no change seen in salivary lactobacilli counts among the control group (table II).

Regarding salivary candida albicans there was statistically significant reduction of counts was registered after ten day consumption of probiotic ice-cream among probiotic group as compared with baseline with p value 0.005(table 2) and also there was statistically significant reduction in candida albicans count among probiotic group as compared control group (p vales =0.034) (table I). There was no difference in the candida albicans count after intake of control ice- cream among nonprobiotic group as compared with baseline (table II).

Table I: salivary streptococcus mutans, lactobacillus and Candida albicans scores at baseline and at follow up intake of probiotic ice-cream and control ice-cream between case and control.

STREPTOCOCCOUS MUTANS		CASE	CONTROL	Mann-Whitney test value	p=value
	BASELINE	48.90± 27.094	24.50± 15.508	35.500	0.272 (NS)
	FOLLOW UP	70.10 ± 48.469	78.70 ± 39.975	2.000	0.00 (S)
LACTOBACILLUS	BASELINE	42.80 ± 18.967	51.60 ± 23.632	46.000	0.762 (NS)
	FOLLOW UP	73.80 ± 24.961	73.20 ± 26.545	27.000	0.082 (NS)
CANDIDA ALBICANS	BASELINE	114.70 ± 31.362	58.10 ± 25.601	47.500	0.850 (NS)
	FOLLOW UP	80.20 ± 17.968	82.50 ± 18.969	22.000	0.034 (S)

Table II: Salivary Streptococcus Mutans, Lactobacillus and Candida albicans scores at baseline and at follow up. Intake of probiotic ice-cream and control ice-cream among case and control.

Streptococcus mutans		**Baseline**	**Follow up**	**Wilcoxon signed ranks test values**	**p=value**
	Case	48.90± 27.094	70.10 ± 48.469	-2.805	0.005 (S)
	Control	24.50± 15.508	78.70 ± 39.975	-1.738	0.082 (NS)
Lactobacillus	Case	42.80 ± 18.967	73.80 ± 24.961	-2.397	0.017 (S)
	Control	51.60 ± 23.632	73.20 ± 26.545	-0.476	0.634 (NS)
Candida albicans	Case	114.70 ± 31.362	80.20 ± 17.968	-2.805	0.005 (S)
	Control	58.10 ± 25.601	82.50 ± 18.969	-0.204	0.838(NS)

IV. Discussion

Dental caries is one of the most prevalent diseases in humans, second only to the common cold. Although the affliction is not life threatening, it causes nagging pain and thus possesses physical as well as psychological discomfort. The economic burden of the disease is also very high. The changes in the homeostasis of the oral cavity with an overgrowth of Streptococcus mutans, lactobacillus and candida albicans is recognized as the primary cause of the disease. Most treatments are now aimed at either elimination of this bacterium or suppression of its virulence.[6]probiotic therapy is one of the promising preventable factors that have been a topic of intense debate.

Commonly, most of the species ascribed as having probiotic properties belong to the genera Lactobacillus and Bifidobacterium. Those bacteria are generally regarded as safe (GRAS) because they can

reside in the human body causing no harm and, on the other hand, they are key microorganisms in milk fermentation and food preservation and used as such from the dawn of mankind.[2, 7] Certain strains of lactobacilli can adhere to the mucosal epithelium, and may thereby compete for adhesion sites with Candida (Strus et al., 2005). In addition, Lactobacillus species produce different metabolites, such as hydrogen peroxide (Strus et al., 2005), and antifungal cyclic peptides (Ström et al., 2002), which inhibit the growth of Candida. The same beneficial effect has been observed in cases of oral Candidiasis infections.[8, 9] Bifidobacteria Bifidum are dominant in the gut flora of breast fed babies. The effect of bifidobacteria is by displacement of proteolytic bacteria causing the disease. Although studies have been performed to validate the survival and positive effects of within in the human body, including immune response and gastrointestinal health in young childrens[10, 11], on the present study species Bifidobacterium Lactis BB-12 and lactobacillus acidophilus but the research concerning on dental health is more limited.

For ethical reasons, legally incompetent subject such as minor child were not included in the present study at this early step, as only few studies have been carried out to examine the possible effects of Bifidobacterium Lactis BB-12 and lactobacillus acidophilus on caries associated microorganisms in the oral cavity, but the encouraging findings may call for an intervention study in the most interesting target group, namely caries prone children. The counts of salivary streptococcus mutans, lactobacillus and candida albicans were estimated using conventional laboratory methods with selective media that correlate well with chair –side test[12].

The results of the present study were in full harmony with study done by Caglar etal [1] using Bifidobacterium lactis Bb-12, in ice cream and Bifidobacterium DN 173010 in yogurt.[12] An invitro study done by Mehanna etal showed that there was inhibition of growth of streptococcus mutans by Bifidobacterium Lactis.[7] There was a reduced candida albicans count from the oral cavities of mice following oral administration of Lactobacillus acidophilus seen with the study done by Elahi etal which is similar to the results of present study.[8]

Lactobacillus acidophilus is a known cariogenic micro organism. This study demonstrated a significant increase in lactobacillus counts in the probiotic group after the test period as compared to control group; this rise can be explained due to the fact that the salivary samples were collected immediately after the consumption of probiotic ice-cream with lactobacillus acidophilus as one of the active ingredient. However with study demonstrating the adhesion of lactobacillus acidophilus on tooth to be transient and with no demonstrable count after one week after cessation, it is least likely to contribute to the cariogenic potential of commercially available probiotic ice-cream.[14]

The mechanism of action of probiotic in oral cavity is not fully understood, but commonly explained by combination of local and systemic immune response as well as non-immunologic defense mechanisms.[15, 16] To be effective against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as a part of biofilm and compete with the growth of dental pathogens [16] . This local event was elucidated by Haukioja et al and Busscher etal in an in vitro study which showed that bifidobacteria and lactobacillus could survive in saliva and adhere to the enamel.[14, 18]

In daily routine, the administration of probiotics to small children may be difficult. The present finding that commercially available ice-cream could serve as a vehicle for probiotic was interesting, the product was well accepted by all participants and no one in the study group could detect the difference between active and the control ice-cream. Thus, there are reasons for believing that commercially available probiotic ice-cream could be a suitable product also for young children.

Current evidence indicates that probiotic effects are strain-specific; therefore, a beneficial effect attributed to one strain cannot be assumed to be provided by another strain, even when it belongs to the same species [2]. A combination of strains can enhance adherence in a synergistic manner[1] hence Bifidobacterium DN 173010 and Bifidobacterium lactis Bb-12 which have proven, to inhibit growth of cariogenic microorganisms, combination of them with Bifidobacterium lactis BB-12 in the commercially available ice-cream can be friendlier to the oral cavity along with gastrointestinal tract.

V. Conclusion

The daily intake of ice-cream containing probiotic bifidobacteria and lactobacillus may reduce the levels of caries-associated streptococcus mutans, lactobacillus and candida albicans in saliva. Further studies are needed to clarify whether this approach is an alternative strategy for the prevention of enamel demineralization.

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