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Original Research

Evaluation of *Lactobacillus* and *Streptococcus mutans* by Addition of Probiotics in the form of Curd in the Diet

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Abstract:

Background: Probiotics are defined as live microorganisms which when taken in adequate amounts provide a health benefit on the host. They have been used to improve gastrointestinal health, and their popularity has prompted increased interest for their role in the promotion of oral health also. The study was undertaken to evaluate the influence of diet alteration on oral microflora with the addition of probiotics in the form of curd in the diet.

Materials and Methods: Sample pool included total 20 children, 15 in experimental group; who were given curd containing probiotic bacteria in their diet daily and 5 children in control group; who were contradicted of food containing probiotics. All the children were followed for over a period of 1-year. A pre and post quantitative analysis of *Streptococcus mutans* and *Lactobacilli* were done in the saliva of both active and control groups, and caries score was recorded. Statistical analysis used: The data thus compiled were statistically evaluated using Chi-square test and Pearson correlation on SPSS software version 14.

Results: In experimental group, 87% children showed decrease and 7% showed increase in *S. mutans* count ($P = 0.83$), whereas 67% showed increase and 7% decrease in *Lactobacilli* count ($P = 0.002$) after 1-year of follow-up.

Conclusions: Probiotics are upcoming as an intriguing field in oral health. Efforts should be made to increase the knowledge of the general dental practitioners with this facet of oral disease therapy and promote the implementation of the concept of "food rather than medicine."

Key Words: Curd, *Lactobacilli*, microorganisms, oral and gastrointestinal health, probiotics, *Streptococcus mutans*

Introduction

Probiotics are considered as live microorganisms which when administered in sufficient amounts bestow a health benefit on the host.[1] They have been used to improve gastrointestinal health, and their fame has aggravated increased interest for their role in the promotion of oral health also. The concept of probiotics developed from Elie Metchnikoff's proposal that the bacteria in fermented products could compete with ailment causing microbes, which are detrimental and health-compromising and thus minimize their ill-effects.[2]

Probiotics can improve patient condition in medical disorders such as diarrhea, gastroenteritis, short-bowel syndrome, inflammatory intestinal diseases (Crohn's disease and ulcerative colitis), cancer, immunosuppressive states, inadequate lactase digestion, pediatric allergies, growth retardation, hyperlipidemia, liver diseases, infections with *Helicobacter pylori* and genitourinary tract infections.[3-5]

Ingestion of probiotics in any type, such as in food substances (cheese, yogurt, fermented milk, fruit juice or chewing gum) or as available in tablets and capsules, has provoked a health gaining effect on oral tissues like reducing the incidence of dental caries, improved management of periodontitis, abridged halitosis and oral candidal infections.[4]

Hence, this study was carried out to access the influence of probiotics in the form of curd, added to the daily diet of the study group to find the effect on the salivary levels of *Streptococcus mutans* and *Lactobacilli* at initial intervention and after 1-year of follow-up.

Materials and Methods

This prospective study was planned and carried out in the Department of Oral Pathology in association with Microbiology Department. The ethical committee clearance was obtained from the concerned authority. A verbal and signed inform consent was obtained from the children and the concerned personages (teachers and parents) were also notified

regarding the same. Government Primary School children aged 10-15 years were examined and a total of 20 healthy children free from any form of mucosal or dental abnormalities and caries were included in the present study, out of which 15 were engaged as experimental group who were given curd containing probiotic bacteria approximately 200 g (1 bowl) in their daily morning diet and 5 as control group who were denied of food containing probiotics over a period of 1-year. The curd was procured commercially from the same manufacturer by the authors and was given to the experimental group. The counts of *S. mutans* and *Lactobacilli* in saliva of these subjects were determined twice by using Dentocult SM Strip mutans and Dentocult Lactobacilli Strip (Orion Diagnostica, Espoo, Finland) at the initial intervention and after a follow-up period of 1-year. Caries scores were recorded before the commencement of the study and after the period of 1-year. To estimate *S. mutans*, children were instructed to swallow excess saliva and then the rough surface of round tipped strip was pressed against the saliva remaining on tongue. The strips were then placed in a selective culture broth supplied by the manufacturer; which were then labeled and incubated in an upright position at 37°C for 48 h. The cap was opened one-quarter of a turn to allow growth of the organisms as per the instructions given by the manufacturer (Figure 1). After incubation, the presence of *S. mutans* was established by identifying light-blue to dark-blue raised colonies on the inoculated area of the strip. The results were assessed according to the manufacturer's guidelines as follows: Class 0: <10,000 CFU/ml, Class 1: <100,000 CFU/ml, Class 2: 100,000-1,000,000 CFU/ml, Class 3: >1,000,000 CFU/ml (Figure 2).

To estimate *Lactobacilli* counts, the early morning saliva samples were collected from all the children. Before taking the samples, children were mandated to avoid eating, drinking and brushing of teeth for 1-2 h. Children were instructed to sit in a relaxed upright position until 1-2 ml of unstimulated whole saliva was collected in the floor of the mouth. They were later asked to spit in a disposable sterile plastic container. Saliva was then poured over the modified rogosa agar surface, screwed tightly, labeled and was placed in an incubator at 37°C for 4 days (Figure 3). The existence of *Lactobacillus* after incubation was evidenced by white to transparent colonies on the modified Rogosa agar surface. The results were assessed as specified by the manufacturer's guidelines as Class 0: 10^3 CFU/ml, Class 1: 10^4 CFU/ml, Class 2: 10^5 CFU/ml, Class 3: 10^6 CFU/ml (Figure 4). After 1-year of follow-up, the saliva samples were again collected from both the groups as previously described. All the data thus accumulated were statistically analyzed using Chi-square test and Pearson Correlation on SPSS software version 14.

Figure 1: Dentocult SM Strip mutans kit and procedure for sample collection.

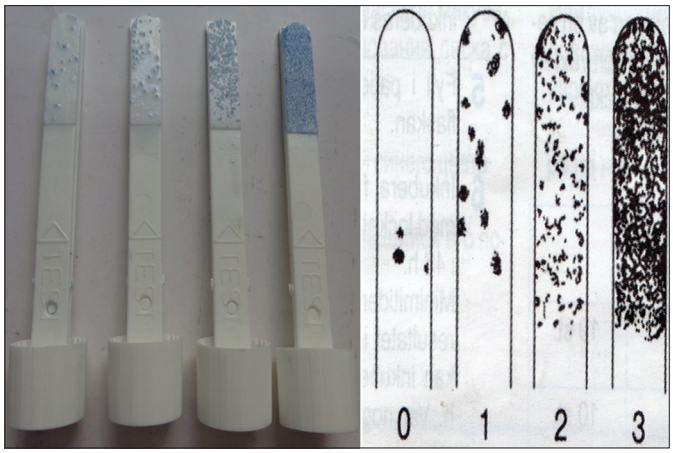

Figure 2: Light-blue to dark-blue, raised colonies on the inoculated surface of the *Streptococcus mutans* strip and manufacturer's guidelines for interpretation of *S. mutans* count.

Figure 3: Dentocult *Lactobacilli* strip kit and procedure for sample collection.

Results

The semiquantitative analysis of *S. mutans* count in experimental group under Class 0, I, II and III at the initial intervention of 0 month showed nil, nil, 5 (33%) and 10 (67%); at 12 months were 2 (13%), 7 (47%), 5 (33%) and 1 (7%), respectively. The semiquantitative analysis of *Lactobacilli* count inactive group

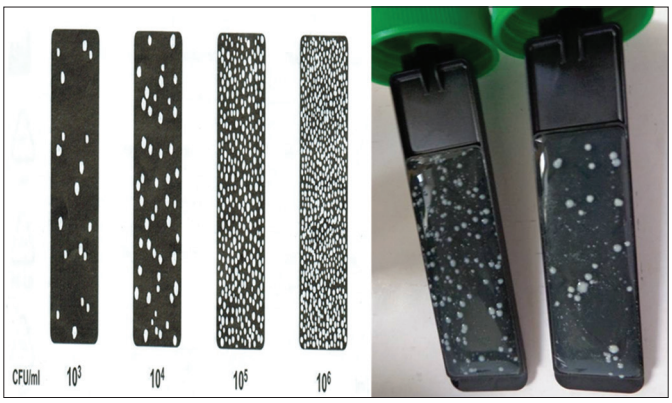

Figure 4: Manufacturer's guidelines for interpretation of *Lactobacillus* count and white to transparent colonies of *Lactobacillus* on modified Rogosa agar surface.

under Class 0, I, II and III at the initial intervention of 0 month showed 2 (13%), nil, 10 (67%) and 3 (20%); at 12 months were nil, 2 (13%), 1 (7%) and 11 (73%), respectively. During the 1-year of follow-up, *S. mutans* decreased in 13(87%) children, increased in 1 (7%), remain unchanged in 1 (7%) children and *Lactobacillus* increased in 10 (67%) children, decreased in 1 (7%), remain unchanged in 4 (26%) children in experimental group (Table 1). *P* value was not significant with $P = 0.832$ for decrease in *S. mutans* (Table 2) and was significant with $P = 0.02$ for increase in *Lactobacillus* (Table 3) after 1-year addition of probiotic in experimental group. Whereas in the control group, within the 1-year period of follow-up, *S. mutans* decreased in 2 (40%) children, increased in 1 (20%) and remain unchanged in 2 (40%) and *Lactobacillus* decreased in 2 (40%) children, increased in 1 (20%) and remain unchanged in 2 (40%). *P* value was insignificant in control group with $P = 0.239$ for *S. mutans* and $P = 0.789$ for *Lactobacillus*. Caries scores remain unchanged in 14 out of 15 children and was increased in 1 child in experimental group with insignificant Pearson correlation $(r) = 0.960$ and significant $P = 0.000$. In control group caries scores was unchanged in 4 children out of 5 and was increased in 1 child with insignificant Pearson correlation $(r) = 0.612$ $P = 0.272$. In experimental group caries score shifted in 1 subject from 0 to 1 in which *S. mutans* count was increased from Class II to Class III over a period of 1-year. In control group caries score shifted in 1 subject from 0 to 1 in which *S. mutans* count was increased from Class I to Class II over a period of 1-year.

Table 1: Semiquantitative analysis of *S. mutans* and *Lactobacillus* in active group.

Class	Number (%) of children			
	At the initial intervention		After 1-year	
	S. mutans	*Lactobacillus*	*S. mutans*	*Lactobacillus*
0	-	2 (13)	2 (13)	-
I	-	-	7 (47)	3 (20)
II	5 (33)	10 (67)	5 (33)	1 (7)
III	10 (67)	3 (20)	1 (7)	11 (73)

S. mutans: Streptococcus mutans

Table 2: Shifting of counts of *S. mutans* in active group after 12 months of follow-up.

S. mutans count at 0 month in active group		Shifting of counts after 12 months of follow-up			
Class	Number of children	Number of children			
		Class 0	Class I	Class II	Class III
0	-	-	-	-	-
I	-	-	-	-	-
II	5	1	1	2	1
III	10	1	5	4	-
P value	0.83				

Table 3: Shifting of counts of *Lactobacilli* in active group after 1-year of follow-up.

Lactobacilli count at initial intervention in active group		Shifting of counts after 1 year of follow up			
Class	Number of children	Number of children			
		Class 0	Class I	Class II	Class III
0	2	-	2	-	-
I	-	-	-	-	-
II	10	-	1	1	8
III	3	-	-	-	3
P value	0.002				

Discussion

Probiotics are microbial cultures or living microorganisms which upon administration in required quantity promote and boost health benefits. An International Life Science Institute Europe consensus document suggested a simple and extensively acknowledged definition of probiotics as "Viable microbial food supplements, which beneficially influence the health of human." These bacteria should belong to the natural flora so that they could resist gastric secretion and survive during intestinal movement.[6]

In India, the occurrence of dental caries in children has been reported to be 87%. In low socio-economic class, the prevalence of caries was reported to be higher (96.2%) than high socio-economic class.[7] A major part of the population in India resides in rural areas (70%) where there is little awareness about the oral health especially for children who have unhealthy eating habits with large quantities of cariogenic products in their diet. For research to be successful in India it has to be directed towards cost effectiveness and easy availability of products for health.[8] The subjects selected in this prospective study were in the 10-15 years age group, which was important for the present study in two-ways. First, this is the period during which permanent teeth are erupting and new surfaces would be colonized by pathogenic bacteria. Any measure directed towards prevention against early colonization might be beneficial in the long-term for the prevention of dental disease. The second reason for selecting this age group was the intellectual ability of the child. In accordance with Jean Piaget at the age of 7 years a child largely corresponds to an increase in cognitive development whereby the child develops

a sense of semi-logical reasoning to infer physical cause-effect relationships.[8] Thus, in this age group a positive compliance could be expected from a child. The subjects selected for the study was on the volunteer basis, those included in the control group was children who disliked milk products, even hated to eat curd and was ready not to consume curd and other fermented products for 1-year whereas the subjects selected for the experimental group was those who liked to have curd. Thus, the large discrepancy was presented between experimental and control group. The parents and teachers were consulted regarding the same and subjects was monitored and regularly followed for 1-year by the authors.

The results of the present study after supplementation of probiotic in the type of curd over a period of 1-year in daily diet in experimental group showed decrease in streptococcus count in 87% and increase in *Lactobacillus* count in 67% of the study subjects. Similar results were found in a study carried by Ahola *et al*., after intake of probiotic in the type of cheese over a period of 3 weeks.[9] In a *in-vitro* study, yoghurt with live bacteria showed selective anti-mutans activity, suggesting that the overall decrease in mutans streptococci *in-vivo* could be due to a bactericidal effect on *S. mutans*.[10] Cagler and Kargula have also shown a reduced *S. mutans* level in the oral cavity of patients receiving fluid or tablet in the form of probiotics.[11] The previous studies present in the literature concerning intervention of probiotics are short-term studies according to us this is the first study carried out for a long period of 1-year. The *Lactobacilli* as a probiotic are believed to have antimicrobial activity and ability to interfere with pathogens on epithelial cells.[12,13] They are also able to stimulate the immune system and alter the pH milieu of the host.[1] When lactic acid bacteria are ingested in milk stuff, the buffering capability of milk hampers the bacterial acidogenity.[14] The availability of calcium and other constituents may also safeguard tooth surfaces and inhibit the attachment of dental pathogens.[15] Standard homemade yogurt refers to those made with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Here in this study, the curd acts as a source of *Lactobacilli*. With reference to word yogurt, it can be suggested that it has been evolved in Turkey as the term "yoghurt" has been derived from a Turkish verb jugurt that means to be curdled or coagulated.[16] Thus from the word curdled the word curd is derived.

Lactobacilli have been used to deliver vaccine components for active immunization *in vivo*.[1] The mechanism of interfering involves the release of bio-surfactants, a structurally diverse group of surface active molecules synthesized by microorganisms and are able to inhibit adhesion of various other microorganisms. In a study carried by Arezoo they showed that the bio-surfactant derived from probiotic bacteria (*Lactobacillus acidophilus, Lactobacillus fermentum* and *Lactobacillus rhamnosus*) could reduce the adhesion of *S. mutans* to the surfaces.[12] Nase *et al*., reported reduced caries in kindergarten children on long-term intake of milk containing the probiotic *L. rhamnosus* CG strain.[17] In order to evaluate whether naturally prevailing oral lactobacilli show probiotic features, *Lactobacilli* were isolated from saliva and plaque from children and adolescents, with or without caries lesions, 23 *Lactobacillus* species entirely repressed the growth of all mutans streptococci tested.[1]

The use of chewing gum containing *Lactobacillus reuteri* as a probiotic bacteria also showed reduction in levels of pro-inflammatory cytokines in gingival crevicular fluid and the use of *Lactobacillus brevis* decreased matrix metalloproteinases (collagenase) activity and various other inflammatory markers in saliva.[18]

The studies so far carried showed short term effects of different formulations of probiotics. Earlier studies have demonstrated a beneficial effect of probiotic lozenges, chewing gums and medical devices on oral health.[19,20] However, none of these formulations is available in India. Though the cost of these probiotic preparations may be within the reach for a population in developed countries, it is beyond the reach of an average Indian. The cost of the probiotic lozenges is about one US dollars, but the curd used in our present study is easily available preparation in the market as well as in almost every home in India. Thus, our study aimed to find a cost-effective and easily accessible alternative to probiotic lozenges and gums.

Manufacturing of curd is an old technique, which dates back to thousands of years, and the knowledge has transferred generation to generation. The word curd is the generalized process of curd making is comprised of modifying the original composition of milk, pasteurizing the yogurt mix, fermentation at thermophilic temperatures (40-45°C) followed by cooling.[16]

Bacteriotherapy in the form of probiotics seems to be a natural manner to uphold health and protect oral tissues from disease due to significant reduction in levels of cariogenic as well as periodontal pathogens.[14]

Conclusion

The small sample size and the large discrepancy between the experimental and control group are the limitation of the study as large sample size could not be adequately followed for such long time. Although authors regularly visited parents and teachers of the study subjects on the regular basis, but there could be chances of error due to long monitoring and follow-up period. Probiotics are transpiring as an interesting field in oral health.[6] There was decrease in *S. mutans* count and increase in *Lactobacillus* count observed in oral microbial flora within the experimental study group after consumption of probiotics in the type of curd over a span of 1-year. However, although the results were insignificant may be due to small sample size but the study could be a pathfinder toward a new concept of food supplements in place of medicines. Efforts

should be made to enlarge the cognizance of the general dental practitioners with this respect of oral disease therapy and persuade the implementation of the concept of "food rather than medicine."[10]

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