A Comparative Study to Evaluate the Effects of Probiotic Curd on Streptococcus mutans, Bifidobacterium dentium, and pH of Saliva in Caries-free Children: An In Vivo Study

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Background: Dental caries is one of the commonest oral diseases that require early stages of prevention and intervention. The primary organisms related to dental caries are streptococcus mutans and lactobacilli. In the past decades, some of the non-pathogenic bacteria called probiotics have been added to the food products for the beneficial effect of human health. Intake of probiotic food products has been shown to have beneficial effects on the oral tissues. Hence the present study aimed to compare the levels of salivary pH, S. mutans, B. dentium counts in children consuming probiotic and non-probiotic curd.

Methods and Material: 40 healthy caries free children were randomly allocated into two groups. First baseline salivary samples were collected from all the participants and tested for salivary pH, salivary B. dentium and S. mutans count. After collecting the baseline values test group of children were provided with probiotic curd whereas the control group were provided with non probiotic curd for 15 days. After 15 days of consumption of these dairy products, salivary samples were collected again and tested for salivary pH, salivary B. dentium and S. mutans count.

Results: Probiotic curd was found to be more effective in reducing the colony counts of B.dentium and S.mutans. There was a marginal decrease in pH in both the groups but was well above the critical pH.

Conclusions: Probiotic curd showed considerable level of decrease in colony counts of B.dentium, S.mutans and salivary pH when compared to normal curd.

Keywords: Bifidobacterium, dental caries, probiotics, Streptococcus mutans

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INTRODUCTION

The oral cavity is a complex ecosystem and harbors diverse array of bacterial species. The equity of these microbes can easily be affected, and an increase in the prevalence of pathogenic organisms can lead to various oral health problems including dental caries.
periodontitis, and halitosis. Dental caries is the most prevalent chronic disease affecting the majority of adults and 60%–90% of school children.[1]

Bacterial species such as *Streptococcus mutans* (SM) and *Bifidobacterium dentium* (BD) are believed to play a crucial role in the initiation of dental caries and *Lactobacillus* in the progression of tooth decay. Despite the use of old conventional, physical, and chemotherapeutic approach for caries management, it failed to prevent further microbial colonization, and their prolonged use is harmful. Diet also plays a key role in caries development. In other words, an anticariogenic diet can be effective in caries prevention.[2] Consumption of live bacteria of human origin is termed bacteriotherapy or replacement therapy. Dairy products such as milk, yogurt, and cheese are used as delivery vehicles for this type of therapy using bacteria. These bacteria must belong to the natural flora to exist in the acid environment during their transit.

The dependence of the good bacteria to the food makes possible measures to restore a healthy microbiome.

Probiotics is termed as “for life”—are live microbial feed supplement that benefits the host by improving its intestinal microbial balance when prescribed with adequate dosages.[3] The most common probiotic strains in food belong to the genera *Lactobacillus* and *Bifidobacterium*.[4]

Probiotics can be made of a single bacterial strain or a group as well. It can be in the form of powder, liquid, gel, paste, and granules. It is available in the form of capsules, sachets, and so on.[3]

World Health Organization (WHO) describes probiotics as live microorganisms, which when consumed in adequate amounts in food or as dietary supplement confer a health benefit to the host.[6] Intake of probiotic-incorporated foods or as a constituent of tablets and capsules, have laid beneficial effects on the oral tissues, such as reducing the prevalence of dental caries, enhanced management of periodontitis, halitosis, and oral candidiasis.

Thus, considering the beneficial effects of probiotic, this study was planned to assess the effects of commercially available probiotic curd (PC) and non-probiotic curd (NPC) on salivary pH and colony forming units (CFU) of SM and BD counts.

**Aim**

This study was aimed to estimate the levels of salivary pH, SM, and BD counts in children consuming PC and NPC.

**Objectives**

1. To compare the salivary pH levels between the PC and NPC group
2. To compare the levels of SM and BD in PC and NPC group
3. To correlate the effect of PC and NPC on levels of SM and BD and salivary pH.

**Materials and Methods**

This randomized controlled clinical trial with two parallel groups was carried out after it was approved by the institutional ethics committee (Ref: VMSDC/IEC/Approval no. 76), Vinayaka Mission’s Sankarachariyar Dental College, Salem, Tamil Nadu, India. Forty institutionalized caries-free children with the age-group of 9–13 years were selected for the study and randomly divided into two groups. All the information regarding the study was given to the parent/guardian in preformed format, and informed consent was obtained.

All children included in the study were institutionalized healthy caries-free children, based on International Caries Detection and Assessment System (ICDAS) scoring criteria, following uniform dietary pattern, neither under antibiotic or probiotic regimen.

Children with decayed, missed, and filled teeth and children under antibiotic treatment, probiotic supplements, and use of xylitol for 3 weeks before and during the course of the study were excluded.

First baseline salivary samples were collected from all the participants by passive drooling method, and salivary pH, salivary BD, and SM count were estimated.

| NPC          | N  | Mean | SD  | t   | P value |
|--------------|----|------|-----|-----|---------|
| Pair 1       |    |      |     |     |         |
| pH (pre)     | 20 | 7.30 | 0.17| 10.50| <0.001**|
| pH (post)    | 20 | 6.75 | 0.25|     |         |
| Pair 2       |    |      |     |     |         |
| *S. mutans* (pre) | 20 | 84.31| 1.99| 92.34| <0.001**|
| *S. mutans* (post) | 20 | 64.59| 2.36|     |         |
| Pair 3       |    |      |     |     |         |
| *B. dentium* (pre) | 20 | 77.05| 2.25| 26.74| <0.001**|
| *B. dentium* (post) | 20 | 59.27| 2.75|     |         |

*P* = level of significance, **values are statistically highly significant
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After collecting baseline values, the test group of children (n = 20) was provided with PC, whereas the control group (n = 20) were provided with NPC far 15 days. After 15 days, salivary samples were collected again similar to the baseline test, and salivary pH, BD, and SM count were evaluated.

Paired t statistical analysis was used to explain this.

RESULTS

In the NPC groups, there was a slight decrease in pH from 7.3 in mean baseline to 6.75 for post values as shown in Table 3. The mean levels of SM reduced from 84.31 to 64.59 after consumption of NPC. Similarily, the mean values of BD counts also reduced from 77.05 to 59.27 after consumption of NPC, which was found to be highly significant with a P = 0.001.

In the PC group, the salivary pH before and after the consumption of PC was 7.24 and 6.66, respectively, as shown in Table 3. The post pH values for NPC and PC reduced from 6.75 to 6.66. Comparison of the salivary colonies of SM for NPC and PC were 84.31 and 83.05, respectively, which was not statistically significant.

Similarly, the mean baseline levels of BD for NPC and PC were found to be 77.05 and 75.75, which reduced to 59.27 and 38.69, respectively. The values also were not statistically significant. The mean levels of SM after the consumption of NPC and PC reduced from 64.59 and 42.74, respectively, which was statistically significant with P value (P = 0.001).

DISCUSSION

Being a multifactorial disease, dental caries is strongly associated with the presence of cariogenic microorganisms, fermentable carbohydrates, and duration of exposure.[7-9]

Considering the antagonistic effect on the growth of cariogenic pathogen, studies have been carried out to evaluate the therapeutic and preventive effects on dental caries, halitosis, candidiasis, and periodontal diseases.[10]

The broad management of dental caries profoundly targets on preventive strategies. Most of the preventive
methods alter the causative factors of dental caries etiology, such as diet, host, salivary pH, and microbial factors.\cite{11}

Probiotic-incorporated food stuffs, such as fruit juice, cheese, yogurt, fermented milk, and chewing gum, or as a constituent of tablets and capsules, have shown beneficial effects on the oral tissues in reducing the incidence of dental caries, improved management of periodontitis, a bridged halitosis, and oral candidal infections.\cite{13}

In this study, institutionalized children were chosen as they were served a uniform pattern of diet in house.

Children who were under any other antibiotic supplements were excluded from this study. Rodgers et al.\cite{13} reported that probiotics work by raising the counts of good bacteria in the gut to combat bad bacteria. Although antibiotics are very effective at attacking bad bacteria, they create a void in destroying both probiotic and pathogenic bacteria after they are used. To retain the favorable effects of probiotic bacteria, subjects who were under antibiotic supplements were excluded in this study.

Study conducted by Featherstone\cite{14} reported that fluoride has the ability to inhibit metabolism after diffusing into the bacteria as hydrogen fluoride molecule when plaque is acidified, to inhibit demineralization, and to enhance remineralization. As this might impair with the results of probiotics, subjects who are under any other fluoride supplements were excluded from this study.

In this study, ICDAS scoring criteria was used as it measures the potential histological depth of various lesions by relying on surface characteristics. This was in accordance with the reports of Stookey\cite{13} who found that ICDAS helps in assessing even the early carious lesion. The benefits of incorporating probiotics in milk products lie in their capability to neutralize the acidic environment in the oral cavity. Curd was selected as it is a dietary constituent accepted by most of the Indian population, easily available, and accepted by all age-groups. The commonly available brand was selected. Unlike other milk products, curd being semisolid can be retained in the oral cavity for a longer period with an extended beneficial effect.\cite{19}

Caglar et al.\cite{17} found a definite reduction in SM after 2-week consumption of yogurt containing the probiotic bacterium Lactobacillus reuteri.

Many types of lactobacillus bacteria are used as probiotics. Lactobacillus acidophilus has the ability to produce hydrogen peroxide and antibacterial substances such as lactocidin and acidophilin. It aids in the production of folic acid, niacin, and pyridoxine. It may also lower serum cholesterol. L. acidophilus has an effective antimicrobial effect against Salmonella, Escherichia coli, Rotavirus, Candida albicans, and Staphylococcus aureus. It is found in small intestine, vagina, urethra, and cervix in both humans and animals, and is present naturally in yogurt.

Since many decades, SM is found to be the main pathogen, which causes dental caries. Other bacterial species such as Bifidobacterium and related genera are aciduric and acidogenic species, routinely isolated on acidic culture media, significantly related to caries experience in children.\cite{16} Only limited studies have reported correlating the levels of Bifidobacterium and dental caries in children.

BD does not simply colonize hard surfaces, and it suggests that surfaces or dentinal components exposed in lesions facilitate the attachment and proliferation of BD. It may therefore be that different factors mediate the establishment of BD and SM in the oral cavity.\cite{18}

Mantzourani et al.\cite{19} identified bifidobacteria levels to be higher in active root carious lesions than inactive root caries lesions in adults. Higher levels were found in occlusal carious lesions in both adults and children than on non-caries surfaces in caries-free individuals.

In this study, salivary samples were collected to assess whether systemic administration of probiotics had any considerable local effects on colony counts of BD, SM, and salivary pH. As saliva is the essential medium contributing to microbial diversity and propagating oral biofilms, any drug administered systemically is first exposed to saliva, which mediates contact with the hard and soft oral tissues.\cite{20}

There was a reduction in both SM and BD counts after the consumption of curd; however, this reduction was more in PC when compared with NPC. This reveals that probiotics incorporated dairy products were effective in reducing the colonies of both SM and BD. However, there was a meager reduction in pH in both groups, which was well within the critical pH.

In this study, PC showed a good decrease in SM colony counts. This was in accordance with the results produced by Sudhir et al.\cite{21} who found a significant decrease in SM colony counts after short-term consumption of PC.

In this study, salivary pH was well within the critical pH of 5.5 with both the curd groups. This indicates that the PC and NPC do not pose any risk of initiation of caries in children. This was supported by the results of Rugg-Gunn\cite{22} who reported that lactose dairy products...
is the least cariogenic of the common dietary sugars and high concentrations of calcium and phosphorous, and the presence of casein in dairy products helps to prevent dissolution of enamel.

Among the two groups, PC showed greater amount of reduction of both SM and BD counts.

This suggests that the effectiveness of PC in children can reduce caries experience. However, further long-term studies are needed to authenticate the role of probiotic dairy products in children with varying degrees of caries risk. Monitoring plaque microbial counts may provide additional indicators of the ecological changes that may occur with the use of these probiotic dairy products.

SM and BD counts and pH changes in saliva play a crucial role in the development and progression of dental caries. Hence, probiotic dairy products can be used as a part of dietary modification for children with varying risk for dental caries.

Further studies can be carried out considering the following factors such as levels of fluoride in drinking water, tooth paste used, and other environmental factors.

**CONCLUSION**

The following conclusions were drawn from the study:

1. A significant decrease was observed in colony counts of SM and BD in both NPC and PC.
2. PC showed considerable level of decrease in colony counts of BD and SM and salivary pH when compared to NPC.
3. A decrease in pH was observed with the intake of PC in the test group and normal curd in the control group but was well within the critical pH.

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**Conflicts of interest**

There are no conflicts of interest.

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