RESEARCH ARTICLE

Prebiotics—A Primeval Measure to Combat Dental Caries: A Short-term Clinical Study

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Abstract

Background: Prevention of dental caries is paramount in reducing the global burden of the disease. The consumption of probiotics as a prophylactic and therapeutic agent for dental caries, has certain limitations. Prebiotics are nondigestible food ingredients, that enhance the growth and activity of probiotic microorganism, thereby help in the establishment of a healthy oral environment.

Aim: The aim of this study was to evaluate and compare the effect of prebiotics, probiotics, and synbiotics on the salivary *Streptococcus mutans (S. mutans)* counts and salivary IgA concentrations.

Methodology: Children of age-group 6–9 years with DMFT score of 5 and above were divided into three groups of 10 each: Group 1 (prebiotics), Group 2 (probiotics), and Group 3 (synbiotics). The functional food therapy was done for a period of 1 month twice daily. The *S. mutans* count and IgA concentrations were assessed pre- and postintervention. The obtained results were subjected to statistical analysis.

Results: A statistically significant reduction of *S. mutans* was seen in all three groups after 1 month. However, no statistically significant difference was noted between the groups.

Conclusion: Prebitoics can serve as an unfortified and natural means of combating dental caries.

Keywords: Immunoglobulin A, Prebiotics, Probiotics, *Streptococcus mutans,* Synbiotics.

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Introduction

Dental caries is a prevalent disease globally. The prevalence ranges from 49 to 83% across different countries, according to Global oral health data bank.[1] Dental caries is considered as a chronic microbial disease with complex etiology. Though reduction in dental caries is one of the objectives in the Global Goals 2020, due to the increasing trend in prevalence, especially in developing countries like India, much research is being directed toward reducing the cariogenic flora from the oral cavity.[2]

Eli Metchnikoff, the Russian Nobel prize winner was the first one to recognize the beneficial role of select bacteria on gastrointestinal tract of humans. Microorganisms have been used since decades to promote health and their use can be mapped out back to the classical Roman literature, wherein foods containing microorganisms have been used as a therapeutic agent. Ever since, much research has been conducted to understand the role of food components and nutrients, which has led to the discovery of Functional foods.[3] The Functional Food Centre in 2012 defines functional foods as "Natural or processed foods that contain biologically active compounds; which in defined amounts provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease."[4]

The term probiotic ("for life") originates from the Greek language. The currently used consensus definition of probiotics was put forth by WHO and FAO of the United States (2001) as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."[5] The of probiotic strains for the prevention of oral diseases, including caries has been reviewed and reported. Probiotics play a major role in restoring the natural saprophytic microflora against the invasion of pathogens, which is considered to be the major etiological factor in oral diseases such dental caries and periodontal disease. Experimental studies using different strains of bacteria such as *Lactobacillus rhamnosus* GG, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus brevis* CD2, *Bifidobacterium* spp., *etc.* have been conducted to evaluate dental caries and its related risk factors, and the results show a favorable reduction in mutans streptococci and lactobacilli count along with plaque pH control.[7-9]

Prebiotics are mostly dietary fibers that are nondigestible. They aid in the selective stimulation of the growth and/or activity of potentially beneficial microorganisms thus producing favorable effects upon host health. Prebiotics have now been proposed for infection control on skin, pH regulation in the body, and to optimize healthy oral microflora. Lactose, Inulin, fructo-oligosacccharides, galacto-oligosaccharides, and Xylo oligosaccharides are the commonly known prebiotics. Certain foods such as bananas, asparagus, garlic, tomato, and onion are some of the rich sources of prebiotics.[11]

Synbiotics are derived by combining prebiotics and probiotics to overcome the possible survival difficulties for probiotics, and were first introduced by Gibson in 2008. Human consumption of

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synbiotics have various health benefits such as, increased levels of lactobacilli and bifidobacteria, improvement of immune-modulating ability, and prevention of bacterial translocation as well as reduction in the incidence of nosocomial infections.[12]

Due to paucity of literature on the role of prebiotics and synbiotics in the oral cavity, the aim of this study was to evaluate and compare the effect of probiotics, prebiotics, and synbiotics on the salivary *S. mutans* counts and salivary IgA concentrations.

Study Design

The study was conducted in a children's orphanage which had children aged 6–10 years. A written informed consent was taken from the caretaker. As there exists a positive correlation between caries experience and salivary *S. mutans*, children with a DMFT score of 5 and above were randomly divided into three groups of ten each: Group 1 (Prebiotics), Group 2 (Probiotics), and Group 3 (Synbiotics).

Prebiotic group was given 100 gm of red banana which contains 40 gm of oligosaccharide. The probiotic group received 100 gm of yoghurt. The synbiotic group received both the banana and yoghurt. The children were instructed to consume their respective functional food during their morning and evening snack break for a period of 1 month. No dental treatment was performed during the study period except for emergency procedures. Oral hygiene instructions and brushing technique was demonstrated however, no dental treatment was performed on the children except for emergency procedures.

Method of Collection of Saliva Sample

An amount of 2 mL of unstimulated saliva from each participant was collected in a labeled sterile container held near the mouth. As the salivary flow rate and composition varies during different hours of the day, the saliva samples were collected between 07:30 and 08:30 a.m. for all subjects. In order to minimize inter individual variation effects which can occur during saliva stimulation process, unstimulated saliva samples were collected.[13] The first sample of saliva was collected prior to the start of the functional food therapy and the second sample was collected after a month. The samples were stored in an ice box at 4°C till evaluation. The *S. mutans* count and salivary IgA concentrations were assessed both before and after the intervention by the following methods respectively.

Method of Salivary Sampling for Estimating *S. mutans*

Mitis salivarius (MS) agar was sterilized by autoclaving at 121°C and at 15 lbs for 15 minutes. After cooling it to 40 to 50°C, 1% potassium tellurite and 0.2 µ/ml of sterile bacitracin solution was added to make the solution selective for *Streptococcus*. In a sterilized Laminar Air Flow chamber, approximately 20 ml of the prepared media was poured into sterile disposable petri plates and allowed to solidify. The collected (saliva) samples were then serially diluted to 10^{-5} using sterile water. Since the bacterial load of the sample was unknown, 100 µl of the undiluted sample, 100 µl of 10^{-2} and 10^{-5} dilutions were inoculated on the media by spread plate technique and incubated at 37°C overnight. The number of bacterial colonies grown on the media were counted and tabulated. The obtained result was subjected to paired t-test ANOVA statistical analysis (Graph 1).

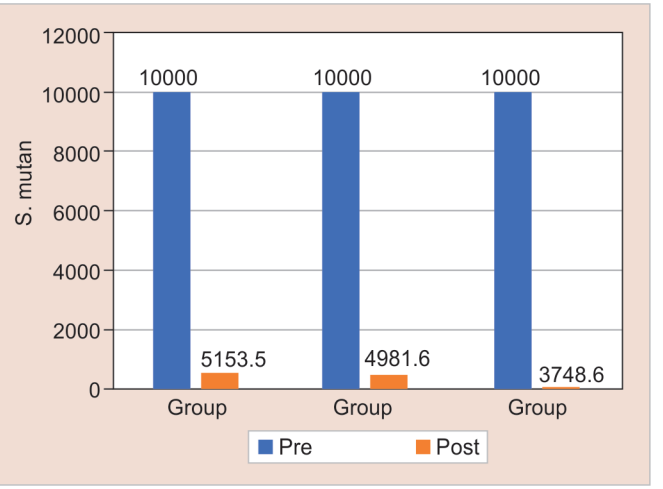
Method of Salivary Sampling for Estimating Immunoglobulin A

The ELISA procedure was followed as per the manufacturer's (Elabscience, Texas, USA) instructions. An amount of 100 µL standard/sample was added to each designated well and incubated for 90 minutes at 37°C. The liquid was removed, and then 100 µl Biotinylated Detection Antibody was added and incubated for 1 hour at 37°C. The supernatant was aspirated and washed thrice. After which 90 µl Substrate Reagent was added and incubated for 15 minutes at 37°C. Postincubation, 50 µl stop solution was added and absorbance was read at 450 nm immediately and results were calculated. The obtained result was subjected to paired t-test ANOVA statistical analysis (Graph 2).

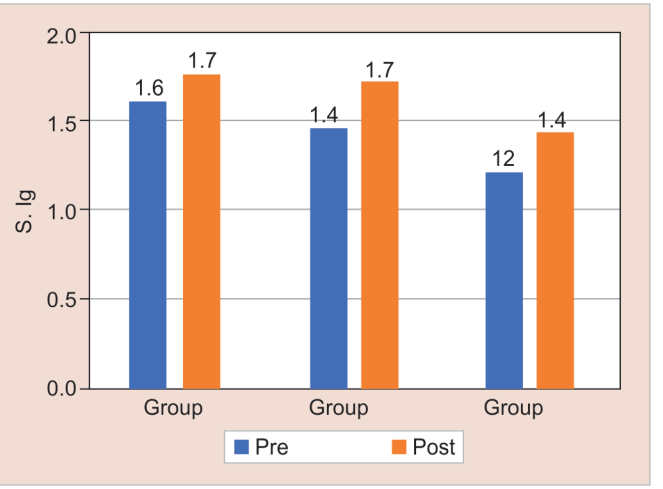
Results

The pre- and postintervention counts of salivary *S. mutans* are given in Table 1. The mean salivary levels of *S. mutans* at baseline were kept at a standard value of 100,000 CFU/ml. The study revealed statistically significant reduction in *S. mutans* counts after the therapy in all three groups. Although the synbiotic group showed the highest reduction of 96,251 CFU/ml, on intergroup comparison there was no statistically significant difference between the groups as shown in Table 2.

The concentration of salivary IgA increased after the intervention in all the three groups, but the increase was found to



Graph 1: Pre-and post-*S.mutans* count across the three groups, Group 1 = Prebiotic, Group 2 = Probiotic, Group 3 = Synbiotic



Graph 2: Pre-and post-salivary IgA concentration across the three groups, Group 1 = Prebiotic, Group 2 = Probiotic, Group 3 = Synbiotic

Table 1: Comparison between pre- and post-mean *S mutants* counts across various groups

	Pre		*Post*		*Mean diff*	*t*	*p*
	Mean	*SD*	*Mean*	*SD*			
Group 1	100,000	0	5,153.5	1,820.4	94,846.5	164.7	0.0001
Group 2	100,000	0	4,981.6	1,598.6	95,018.4	188.02	0.0001
Group 3	100,000	0	3,748.6	1,981.8	96,251.4	153.59	0.0001

Test applied paired *t* test
Inference—Significant reduction in *S mutants* counts seen post-intervention across all the three groups and the difference is found to be statistically significant.

Table 2: Mean reduction of *S. mutans* was highest in group 3 followed by group 2 and the least was observed in group 1

	Mean	*SD*	*F*	*p*
Group 1	94,846.5	1,820.5	1.8	0.1
Group 2	95,018.4	1,598.1		
Group 3	96,251.4	1,981.7		

Test applied ANOVA
Inference—Mean reduction of *S. mutans* was highest in group 3 followed by group 2 and the least was observed in group 1, the mean difference was not statistically significant.

Table 3: Mean comparison between pre- and post-mean S.IgA across various groups

	Pre		*Post*		*Mean diff*	*t*	*p*
	Mean	*SD*	*Mean*	*SD*			
Group 1	1.61	0.38	1.76	0.53	-0.53	-0.91	0.1
Group 2	1.46	0.29	1.72	0.23	-0.25	-1.66	0.05*
Group 3	1.21	0.26	1.44	0.22	-0.23	-2.04	0.05*

*p >0.001
Test applied paired *t* test
The concentration of salivary IgA increased after the intervention in all the three groups, but the increase was found to be statistically significant in group 2 and 3

be statistically significant only in the probiotic and synbiotic group as shown in Table 3.

Discussion

Dental caries is a multifactorial disease with numerous well-known components which collectively contribute for the occurrence of the disease. However, *S. mutans* is considered to be the major organism contributing to this over-whelming disease. The outcome of the present study revealed a significant reduction in the salivary mutans levels in all the groups after a period of 1 month (Table 1) with no statistically significant difference between them (Table 2).

Lactobacillus acidophilus was the probiotic strain used in this study. Ferrazzano et al. suggested that the vehicle for administration of probiotics should be of milk origin due to the presence of casein phosphopeptides that have an inhibitory effect on demineralization and promote the remineralization of dental enamel.[14] Dairy products supplemented with probiotics are a natural means of oral administration and can be easily adopted in the dietary regimen of children. Hence, the probiotic used in the present study was in the form of yoghurt. Probiotic organisms are known to have a positive impact on oral health. They compete with oral pathogens for adhesion sites by transforming the composition of salivary pellicle and are also involved in metabolism of substrates causing a depletion of nutrition. They also have the ability to adhere to epithelial cells and produce several antimicrobial substances against pathogenic microorganisms.[14] In our study, a significant decrease in *S. mutans* count was seen after a month of probiotic administration suggesting that the probiotic bacteria *L. acidophilus* was effective in reducing the *S. mutans* count in saliva. Several other studies conducted by Bafna et al., Lin X et al., Cildir et al., and Singh et al. have also reported similar results.[14–17]

In the present study a statistically significant increase in concentration of salivary IgA was noted in the probiotic group (Table 3) at the end of the study period which indicates that *L. acidophilus* exerts an immunostimulatory effect by enhancing the sIgA production. Similar results were also obtained in previous studies, where probiotic administration improved the oral immune system.[18,19]

Prebiotics enhance the growth and activity of the beneficial probiotic organisms thereby modifying the balance of the microflora. The characteristic feature of prebiotic ingestion is that they are resistant to digestion and are not broken down by salivary and gastric enzymes, and pass unchanged into the large intestine, where they are then selectively fermented by beneficial microorganisms. The by-products produced by prebiotic fermentation forms the substrate for another microorganism, and this process is termed as "cross-feeding." Fermentation of prebiotics by gut microbiota results in the production of short-chain fatty acids (SCFAs), including lactic acid, butyric acid, and propionic acid. These SCFAs have multiple beneficial effects on the body such as decreasing the gut pH, promoting epithelial development and

enhancing the activity of macrophages. As SCFAs have the ability to diffuse into the blood stream through enterocytes, prebiotics have the potential not only to affect the gastrointestinal tract but also distant site organs.[20]

As red banana contains a high concentration of fructo-oligosaccharide (40 gm/100 gm) it was selected as the prebiotic of choice in the present study. The prebiotic group revealed a significant reduction in salivary mutans levels after 1 month (Table 1) suggesting that prebiotics by itself can be used in eliminating the cariogenic streptococci. The antibacterial effect of the prebiotics is due to the mechanism by which they bind to the pili of microorganism thereby inhibiting their attachment on host surface. They also enhance the activity of lysozyme enzyme which acts on the peptidoglycan layer of the bacterial cell wall causing cell death.[11] With respect to the salivary IgA, although an increase in the concentration was seen in the prebiotic group after a month (Table 3) it was not found to be statistically significant. Contrary to the result of our study, in an experiment done by Nakumara et al, dietary fructo- oligosaccharides was seen to increase the intestinal IgA response in the small intestine of infant mice.[21] Prebiotic supplementation increased fecal secretory IgA and postnatal immune development in infants over a 32-week period.[23]

The synergism of prebiotics and probiotics as synbiotics is said to have an added benefit to furnish superior oral health conditions.[2] The rationale behind the development of synbiotics is that, probiotic microorganisms may not have the oral cavity as their innate habitat, thereby reducing their likelihood to confer a health benefit. Hence by the addition of a prebiotic substrate, these microorganisms can survive better and become tolerant to changes in pH, temperature, and oxygen.[25] However, in our present study, although a significant decrease in *S. mutans* was noted in the synbiotic group (Table 1), the mean differences in the reduction of mutans streptococci was not found to be statistically significant between the groups (Table 2). These results reveal that prebiotics need not necessarily be combined with probiotics to bring about a therapeutic effect. Prebiotics, independently and unaided have proven to be efficient in eliminating the cariogenic microorganism.

The consumption of probiotics is noted to cause complex infections in immuno-compromised individuals.[23] Probiotics may also be responsible for production of harmful metabolites, excessive immune stimulation in susceptible individuals, gene transfer and they may also harbor transmissible antibiotic resistance determinants.[24] Considering the limitations in the use of probiotics, prebiotics incorporated in the daily diet, could serve as a valuable tool in caries reduction as they are completely safe with no undesirable effects on the host health. As this is a pioneer study which evaluated the effect of prebiotics and synbiotics in the oral cavity, more long-term studies are required to substantiate the role of these functional foods on dental caries.

Conclusion

Prebiotics, can serve as an innate, unfortified, and organic approach to combat dental caries and has shown drastic reduction in the cariogenic microorganism. It eliminates the tedious processing methods which goes into the manufacturing of probiotic foods. Prebiotics offer a simple, safe and an economical regimen to prevent dental caries. The present study establishes a foundation for the use of prebiotics, that has the potential to revolutionize the concept of caries prevention.

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