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Original Research Article

Effectiveness of probiotic toothpaste in reducing streptococcus mutans in plaque around orthodontic brackets

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ABSTRACT

Introduction: The present study was done to evaluate and compare the effect of probiotic toothpaste in reducing streptococcus mutans and to improve periodontal status of patients who are undergoing fixed orthodontic treatment.

Materials and Methods: A total of 60 participants undergoing fixed orthodontic treatment in age range of 14-29 years were selected to receive probiotic toothpaste for a 30 days period. Participants were examined at 2 time points: base line (start of the study) T0 and after 30 days (T 1) of using probiotic toothpaste. In both the groups, S. Mutans Colony Count and Orthodontic Plaque Index was evaluated.

Result: At T0, the mean S. mutans colony count was found to be 63.31± 27.23x10-2 CFU/ml. At T1 the mean S. Mutans colony count was found to be 29.70 ± 15.41x10-2 CFU/ml. The mean Plaque Index was found to be 1.23+0.53 at T0. After using probiotic toothpaste twice daily for a period of 1 month (T1) the mean Plaque Index was found to be 0.11±0.32.

Conclusion: Use of probiotic toothpaste twice daily has been found useful in reducing S. Mutans colony count after using for a period of 1 month.

1. Introduction

White spot lesions are caused by S. Mutans and are common on teeth during and after orthodontic treatment. The complex design of orthodontic bands and brackets can create an ecological environment that promotes colonization and growth of cariogenic S. Mutans. They are most commonly found in the cavities and crevices of teeth. They abundantly colonize plaque biofilms in the oral cavity. One of the virulence factors of S. Mutans in caries development is its ability to adhere to tooth surfaces and form biofilms.[1] They grow and synthesize dextran capsules that bind to tooth enamel and form biofilms. It demineralizes tooth enamel, causing tooth decay and bacterial infection of the teeth.[2] These bacteria are more common in orthodontic patients than in non-orthodontic patients. They are most common around the gingival region of bands of the posterior teeth, but anteriorly, the lateral incisors, then the canines, of both arches are most commonly affected. The maxillary lateral incisors and canines accumulated the highest level of dental biofilm, which may be because these teeth are located at the corners of the mouth and receive relatively less tooth brushing strokes during oral hygiene.[3] To inhibit or reverse enamel demineralization, various methods have been proposed such as fluoride delivery systems, casein phosphopeptides, amorphous calcium phosphate, and argon laser enamel surface attenuation. Continuous fluoride release from

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fluoride-containing sealants, elastomeric strands, primers, and adhesives has also been found useful.

Often the application of fluoride not only requires frequent visits to the dentist but also causes discoloration of the teeth, leading to decreased esthetics. Biological methods such as antibiotics, antimicrobial therapy with chlorhexidine, povidone iodine, fluoride, and penicillin have gained importance in recent years. The application of broad spectrum antibiotics and antimicrobial therapy can suppress the caries infection but never totally eliminate it.[4] This disadvantage led to the idea of using probiotics as an alternative. The idea of probiotics dates back to the first decade of 1900s when the Ukranian bacteriologist and Nobel Laureate Metchnikof (1908) studying the flora of the human intestine developed a theory that senility in humans is caused by poisoning of body by the products of some of these bacteria. To prevent the multiplication of these organisms he proposed a diet containing milk fermented by lactobacilli, which produce large amounts of lactic acid that could increase the life span of humans.[5]

Several studies have concluded that there are positive effects of probiotics on gingivitis, periodontitis, halitosis, and candidiasis as well along with their anticaries effect. Studies have established that the level of S. Mutans is reduced after the use of probiotics. However, few studies have been conducted examining the effects of topical administration of probiotic agents such as mouthwashes, lozenges, cheese, milk chewing gum, and yogurt.[6,7] The aim of this study was to evaluate the effect of probiotic toothpaste in reducing S. Mutans and improving periodontal health in patients undergoing fixed orthodontic treatment.

2. Materials and Methods

This prospective clinical trial was conducted at the Department of Orthodontics and Orthodontics, Swami Devi Dyal Hospital and Dental College, Barwala, Panchkula. Microbiological analysis were performed at the Microbiology Department of Swami Devi Dyal Hospital and Dental College Barwala Panchkula. Ethical approval for this study was obtained from the Institutional Review Board of Swami Devi Dyal Dental College, Barwala. (Figure 1)

2.1. Study design

A total of 60 participants in age range of 14-29 years were selected to receive probiotic toothpaste for a 30 days period. Participants were monitored throughout this time period. The sample was calculated by the formula:

$N = \frac{2(Z^2 - S^2)}{d2}$

z = 1.96(95% CI)

where s = 0.34 (standard deviation of outcome)

d = 0.12 (mean difference of mean)

2.2. Oral hygiene instructions

Participants were instructed to maintain the current standard of care for oral hygiene, namely brushing twice daily. Patients were instructed to brush their anterior teeth up and down and their posterior teeth in circular motions for 2 minutes. As an exception participants were instructed not to brush their teeth before the appointments or upon arrival to the Department of orthodontics Swami devi dyal hospital and dental college Barwala.

2.3. Participants selection criteria

Patients within the age group of 14 to 29 years undergoing fixed orthodontic therapy on both arches with attachments on atleast 20 teeth including bonded/banded first molars for a minimum of 3 months; Patients had fully erupted all teeth prior to second molars; Caries inactive prior to study initiation; Healthy systemic condition; Non smokers and did not consume alcohol and Habit of brushing twice daily. Patients using any antimicrobial mouth rinses, probiotics (unrelated to the study) antibiotics or anti-inflammatories medications within one month prior to study were not included.

2.4. Data collection

Participants were examined at two times: 0 days; T 0 (baseline) Probiotic started, and 30 days (T1, discontinuation of probiotic toothpaste). The toothpaste used was Purexa Probiotic Toothpaste, containing Bacillus Coagulans (probiotic), fluoride and xylitol as key ingredients. Participants were instructed to use estimated pea sized dab of toothpaste. Patients were moniored with the help of compliance monitoring.

2.5. Compliance monitoring

In order to both assist and monitor compliance, participants were instructed to mark daily on the calender two times when they brushed their teeth with probiotic toothpaste. This was basically to remind the participant to brush daily with toothpaste and to increase their compliance. Percentage of 70% or higher meant complaint patient and those with a percentage lower than 70% were identified as non compliant.

The following clinical and microbiological data were collected at each visit: S. Mutans Colony Count and Orthodontic Plaque Index

2.5.1. Assessment of S mutans colony count

At each time interval, the elastomeric module was carefully removed and the archwire was released by the same operator. Using the technique described by Pellengrini et al,[8] (four pass technique), a sterile scaler was used to collect plaque samples from all the four labial surfaces (ie. mesial,

distal, gingival and occlusal) immediately around the upper lateral incisor orthodontic brackets.

Plaque specimens were collected in screw-cap vials containing 10 ml of 10% sterile thioglycollate broth to serve as transport medium. To estimate the number of streptococcal colonies, samples were inoculated into Mitas salvarius bacitracin.

A 1% potassium tellurite solution was added to make the solution selective for streptococci. 100 microliters of thioglycollate broth was transferred under aseptic conditions to sterile Mitis salvarius bacitracin plates. It was then evenly spread over the surface of the medium using a sterile dispenser. After 10 minutes, the Mitis salvarius bacitracin plate was incubated at 37 degrees Celsius for 48 hours with the addition of 5 percent carbon dioxide. Colonies of streptococci were counted with a digital colony counter.

2.6. Assessment of periodontal status

Orthodontic Plaque Index (OPI):[9] The Orthodontic Plaque Index was published in 2012 by Katrin Beberhold et al. It was developed with special circumstances in mind when assessing oral health during orthodontic treatment. OPI visually records the presence of plaque around the multi-bracket appliance by staining the tooth or surface with a plaque visualization solution. Teeth with orthodontic bands are not included. In clinical applications of OPI, the dentition is divided into sextants. (Table 1)

OHI is given as a score from 0 to 4. Assess the degree of plaque accumulation on both sides of the bracket base (mesial, distal, occlusal/incisal, cervical) and the condition of the adjacent marginal gingiva.

0: No plaque deposits on the tooth surfaces surrounding the bracket base.

1: Plaque deposits on one teeth surfaces at the bracket base.

2: Plaque deposits on two tooth surfaces at the bracket base.

3: Plaque deposits on three tooth surfaces at the bracket base.

4: Plaque deposits on four tooth surfaces at the bracket base and/or gingival inflammation indicators (plaque deposits near the gingiva do not necessarily have to be present)

Data collected in this study were obtained from the Statistical Package of Social Science (SPSS) using version 23.0. Comparisons was performed by applying the specific Wilcoxon signed-rank test and the significance level was set at P-value < 0.05.

3. Results

3.1. Evaluation of S mutans colony counts

The method was performed manually by quantitative differential culture at the beginning (T0) and at the end of 30 days (T1).

Fig. 1: Armamentarium

Fig. 2: Plaque sample collected

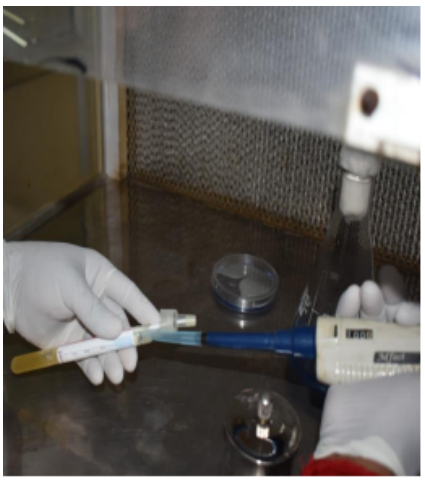

Fig. 3: Thioglycollate broth transport medium

Table 2 shows a comparison of S. Mutans colony counts before and after probiotic administration for 30 days. The mean S. Mutans colony count at baseline (T0) was 63.31 ±27.23 x 10^{-2} CFU/mL and after applying probiotic

Table 1: Orthodontic plaque index

Sextant	Description
First/S1	Maxillary molar and premolar area; first quadrant (17-14)
Second/S2	Maxillary canine and incisor area; first/second quadrant (13-23)
Third/S3	Maxillary premolar and molar area: second quadrant (24-27)
Fourth/S4	Mandibular molar and premolar area; third quadrant (37-34)
Fifth/S5	Mandibular canine and incisor area; third/fourth quadrant (33-43)
Sixth/S6	Mandibular premolar and molar area; fourth quadrant (44-47)

Table 2: Comparison of streptococcal colony count.

Time period	N	Mean ± SD CFU/ml (10^{-2})	Standard error mean	Mean difference	Z value	P value
T0 Baseline	60	63.3±24.23	3.51	33.61	-6.73	<0.001
T1 after 1 month	60	29.70±15.41	1.98			

Table 3: Orthodontic plaque index (OPI)

Time period	N	Mean ± SD CFU/ml (10^{-2})	Standard error mean	Mean difference	Z value	P value
T0 Baseline	60	1.23±0.53	0.06875	1.11667	-7.386	<0.001
T1 after 1 month	60	0.1±0.32	0.04179			

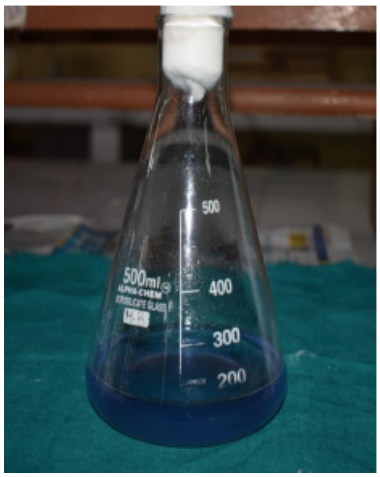

Fig. 4: Sample inoculated

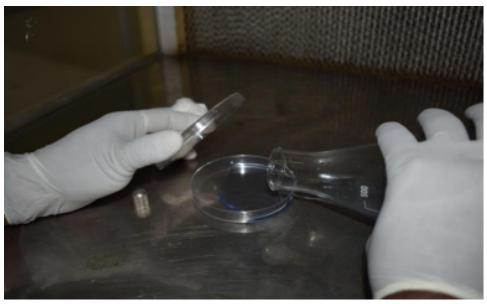

Fig. 5: Mitissalivarius bacitracin plate

toothpaste for one month (T1) was found to be 29.70 ± 15.41 x 10^{-2} CFU/mL. The mean standard error at T_0 was 3.51 and at T_1 was 1.98.

We found the Z-score to be -6.73. This indicates that the raw score is 6.73 standard deviations below the mean. P-value was observed to be <0.001, which is highly significant.

3.2. Assessment of periodontal status

Table 3 shows a comparison of plaque index before and after 30 days of probiotic administration. At study initiation (T_0), the mean plaque index was 1.23±0.53. After using the probiotic toothpaste twice daily for one month (T1), a mean plaque index of 0.11 ± 0.32 was recorded. The mean standard error at T0 was 0.068 and the mean at T1 was 0.

The 041 or Z-score is -7.386, indicating that the raw score is 7.386 standard deviations below the mean. P-value was observed to be < 0.001, which is highly significant.

4. Discussion

The present study was conducted to evaluate the effect of probiotic toothpaste on S. Mutans level in patients undergoing fixed orthodontic treatment. The study was initiated 3 months after orthodontic procedure to rule out a precipitous decline in bacterial counts after bracket application. After applying the probiotic toothpaste twice daily for 1 month, the number of S. Mutans colonies was significantly reduced. At T0, the mean S. Mutans colony count was found to be 63.31 ± 27.23 x 10 -2 CFU/ml. A mean streptococcal colony count of 29.70 ± 15.41 x 10-2 CFU/ml was found in T1. The mean plaque index at T0 was

found to be 1.23 ±0.53. After using the probiotic toothpaste twice daily for one month (T1), a mean plaque index of 0.11 ± 0.32 was recorded.

To overcome the limitations of traditional disease control strategies, many researchers are developing 'probiotics' to treat infections that cause tooth decay. Probiotics are defined by FAO/WHO (Food and Agriculture Organization/World Health Organization) as live microorganisms that, when administered in adequate amounts (either in food or as dietary supplements), confer a health benefit on the host. (improved microbiological balance in intestine tract). Such non-pathogenic organisms (yeasts or bacteria, especially lactobacilli) are present in food and can have beneficial effects on the health of the host. Although probiotics have been used in fermented products for decades, the potential use of probiotics as a medical nutritional therapy has not been officially recognized.[9]

Metchnikoff was the first one to state that consumption of fermented milk with viable bacteria could enhance the longevity of Bulgarian people. The term "probiotic" rather than "antibiotic" was first proposed by Lilley and Stillwell in 1965.[10] The use of probiotics has come a long way since the 20th century. It has been used to modify the microbial flora of the stomach and intestines. Probiotics can create a biofilm that acts as a protective layer of oral tissue against oral disease by keeping bacterial pathogens away from the oral tissue by filling the space for pathogenic bacteria to enter.

Some probiotic species also secrete antibacterial compounds called bacteriocins for example, Reuterin.[11,12] A major drawback of using saliva to estimate the amount of S. Mutans is that S. mutans in saliva represents the total number of microbes in the oral cavity from previous carious lesions, the tongue, and other sites that harbor microbes, and is also unique to the tooth surface. Saliva clearance in anterior teeth is much less when compared to posterior teeth.[13]

SK Cildir et al in 2009[14] had shown that daily consumption of fruit yogurt containing Bifidobacterium animalis subsp lactis DN-173010 could reduce salivary levels of S. Mutans in orthodontic patients using fixation devices. Similar results regarding the use of probiotic yogurt were reported by Jose, Padma Nabhan, and Chithranjan in 2013,[15] Bafna et al in 2017[16] and Megha et al[17] in 2018. Bifidobacterium bacteria and lactobacillus duo is naturally heterofermentative, producing both lactic and acetic acid. Bifidobacteria also have the ability to bind to Fusobacterium nucleatum-coated hydroxyapatite, and lactobacilli have been shown to transiently colonize the oral cavity. These properties of both Bifidobacterium and Lactobacillus confers the ability to adhere to oral mucosa and dental tissues as part of a biofilm whereby inhibiting the growth of dental pathogens to be effective against oral infections. conflict and also explain the observed reduction by S.Mutans count in saliva in the previous study.[17]

Chuang et al,[18] found that the use of probiotic containing tablets did not create a statistical difference in terms of lactobacillus quantities compared with the control group. However the probiotic intervention tended to increase the amount of lactobacillus. They suggested that this increase in lactobacillus level could be because the oral tablet used in their study contained L.paracasei extract which was not considered cariogenic. Studies on use of probiotic mouth rinse by Shah et al in 2018[19] and Goyal et al in 2019[2] showed that daily use of probiotic mouth rinse could reduce the levels of S. Mutan in the saliva in orthodontic patients with fixed appliance which was in concordance to our study.

The antiplaque activity of probiotic mouth rinse may be achieved in several ways such as reducing bacterial adhesion to the tooth surfaces, inhibiting growth and proliferation of microorganisms on the tooth surface inhibiting formation of intracellular plaque matrix, modifying plaque biochemistry to reduce the formation of cytotoxic products and modifying plaque ecology to a less pathogenic flora. Probiotic mouth rinses utilize the natural commensal bacteria to provide a natural defense system against harmful bacteria. Goyal et al in 2018[20] found that the level of P.gingivalis were significantly decreased with probiotic mouthwash hence probiotic mouthwash can be used as a adjunctive measure along with regular brushing to improve periodontal status during fixed orthodontic treatment Amflor mouthwash can effectively be used for white spot lesions with gingival and periodontal problems. Previous studies on the use of probiotic toothpaste by Jose et al[15] in 2013, Majstorvoic[21] in 2013 and Baka[22] in 2018 reported a significant decrease in S.mutans level in the plaque around in orthodontic brackets.

This reduction can be attributed to the formation of a biofilm that prevents adhesion of pathogens, the competitive agents produced by the bacteria.

The topical application of probiotic tooth paste was found to be more efficient in reducing S.mutans levels than probiotic curd but this difference was not statistically significant.[15,16]

5. Conclusion

After applying the probiotic toothpaste twice daily for 1 month, the number of S. Mutans colonies was significantly reduced.

A statistically significant difference in plaque index was observed after using the probiotic toothpaste (P-value was observed to be <0.001). Orthodontists should probably work towards introducing an oral hygiene regimen involving a probiotic dentrifice for routine orthodontic treatment. Since this was a short term study longer period of evaluation would establish the long term advantages of probiotics in orthodontic patients and relative merits of local probiotic therapy.

6. Conflict of Interest

None.

7. Source of Funding

None.

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