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A comparison of the efficacy of a probiotic toothpaste, a fluoridated toothpaste in management of *Streptococci mutans* in plaque around orthodontic brackets- An in vivo study

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ABSTRACT

Introduction: The objective of study was to evaluate and compare the efficacy of Probiotic toothpaste, fluoridated toothpaste and non-fluoridated toothpaste (control) on the *Streptococci mutans* levels in the plaque surrounding orthodontic brackets.
Materials and Methods: The randomly selected patients are divided into 3 groups (20 each) on the basis of type of toothpaste given to them. The patients in groups were asked to brush twice daily with given toothpastes and to discontinue using their normal toothpaste. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors by four passes, each along the tooth at the bracket inter-face at the gingival, mesial, distal, and occlusal aspects. The isolated plaque is sent to Microbiology laboratory soon after collecting in a sterile container. The presence of *S mutans* was evaluated in colony forming unit through culture formation and statistical analysis was performed, and comparisons were made.
Results: After one month application of a probiotic containing toothpaste (group 3), a fluoridated containing toothpaste (group 2) and a non-fluoridated toothpaste(group 1) the level of *streptococcus mutans* significantly reduced in group 2 (non-fluoridated toothpaste) and group3 (Probiotic toothpaste) & minimal reduction is shown in gruop 1. Conclusion: The use of probiotic toothpaste causes a significant decrease in the *S mutans* levels in the plaque around orthodontic brackets in orthodontic patients.

1. Introduction

Advances in orthodontics have improved the quality of appliances and treatment protocols, raising the standard of patient care. However, enamel demineralization is still a problem associated with orthodontic treatment, leading to the formation of white spot lesions; this is a grave concern to orthodontists and patients. The overall prevalence of white spot lesions among orthodontic patients has been reported to be between 4.9% and 84%.[1] A white spot lesion is the precursor of enamel caries. White spot lesions develop as a result of an interrupted process with periods of remineralization and demineralization.[1]

When basic oral hygiene is poor, orthodontic appliances create areas of plaque stagnation, especially around brackets, bands, wires, and other attachments; this facilitates the development of white spot lesions.[1] Levels of acidogenic bacteria, present in the plaque, notably Streptococcus mutans, are higher in orthodontic patients than in non-orthodontic patients.[2–4] This causes demineralization

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around the brackets and leads to white spot lesions. They are most prevalent around the cervical region of bands in the posterior region, whereas in the anterior region, the lateral incisors in both arches, followed by the canines, are most commonly affected.[1]

Biological methods such as antibiotics, antimicrobial therapy with chlorhexidine, povidone iodine, fluoride, and penicillin have gained importance in recent years.[5] The application of broad-spectrum antibiotics and antimicrobial therapy can suppress the caries infection but never totally eliminate it.[5] None of these medicaments has been able to successfully preclude the regrowth of residual pathogens or reinfection from external sources; this means that antibiotic and antimicrobial therapies must be given at regular intervals for effective long-term results.[5]

At the turn of the 20th century, Elie Metchnikoff, a Nobel Prize-winning Russian, made the revolutionary discovery of probiotics. Probiotics are "live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance."[5]

A few studies have evaluated the effects of local administration of probiotic agents such as mouthwashes,[6] lozenges,[7] tablets, straws,[8] milk,[9] cheese,[10] ice cream,[11] chewing gums,[12,13] yogurt,[14,15] and other supplements and have found that these have a beneficial effect on oral health. The benefits on oral health in preventing gingivitis, halitosis,[16,17] and caries[14,15] have been recognized, and thus probiotics have been incorporated into mouthwashes and dentifrices for popular consumption. Some studies have established that the level of *S mutans* in saliva is reduced after the use of probiotics; this would be beneficial in orthodontic patients also.[14]

Probiotics are commonly consumed as part of the diet in several cultures in the form of fermented foods such as yogurt and soy yogurt, or as dietary supplements with added active live cultures. They have proved to be beneficial in treating malnourishment, lactose intolerance, calcium availability, bowel problems such as constipation, urogenital infections, and atopic diseases such as antibiotic induced diarrhea, and in improving the immune system, alleviating chronic intestinal inflammatory diseases, and preventing and treating pathogen-induced diarrhea.[16]

S mutans concentration in plaque would be more representative of the caries-inducing potential in the anterior teeth where salivary clearance is less effective. Since the localized effect of probiotics on the plaque surrounding orthodontic brackets has not been studied, we conceived this study to evaluate whether probiotic systems are beneficial to orthodontic patients. It is desirable to establish which delivery system is more efficient, and thus this study was designed to compare the efficacy of probiotic toothpaste, fluoridated toothpaste & non fluoridated toothpaste. Our aims were to evaluate and compare the effect of probiotic toothpaste on the *S mutans* levels in the plaque surrounding brackets in orthodontic patients.

2. Materials and Methods

The study was double-blinded and randomized, consisting of 60 randomly selected orthodontic patients having orthodontic treatment. The randomly selected patients are divided into 3 groups (20 each) on the basis of type of toothpaste given to them. Group 1 consisted of 7 male and 13 female patients who received non fluoridated toothpaste (Control group). The patients in group 2 consisted of 9 male and 11 female patients were asked to brush twice a day with fluoridated toothpaste to discontinue using their normal toothpaste. The patients in group 3 consisted of 7 male and 13 female patients were asked to brush twice daily with Probiotic containing toothpaste and to discontinue using their daily use toothpaste.

The inclusion criteria for study were Permanent dentition, Good general health (no significant medical history or drug use during the last month). No anti-inflammatory or antibiotic medications taken in the month before the study. No active carious lesion. No chewing gum or mouthwash used in the last week and during the study. Habit of brushing twice daily with toothpaste. Age between 14 and 29 years (average, 20 years).

All the toothpaste were masked (white surgical tape) and delivered to patients, The patients were asked to brush with an up-and-down motion on the front teeth and a circular motion on the back teeth for 2 minutes; this was demonstrated by the same operator. Samples were collected at 2 times: before the study began and after 30 days.

At each time interval, the elastomeric modules were carefully removed to disengage the archwires for collecting samples by operator. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors with a sterilized scaler using a 4-pass technique as suggested by Pellegrini et al.[17]

Four passes, each along the tooth at the bracket inter-face at the gingival, mesial, distal, and occlusal aspects, were used to prevent overloading the instrument tip.

The isolated plaque is sent to Microbiology laboratory soon after collecting in a sterile container.

The plaque sample is diluted in 2ml normal saline and serially diluted. 100 micro liter of the serially diluted sample is plated on selective media Mutans-Sanguis agar and incubated in 10% CO2 at 37 degree C overnight. *Streptococcus mutans* can be differentiated by their characteristic colony morphology and further confirmed by fermentation of Phenol Red Mannitol broth and Phenol Red Lactose Broth. Grams Stain, Catalase Test, 4% NaCl tolerance are also done to confirm the identity. The number of colonies are counted and expressed as Colony Forming Units per milliliter (CFU/ml).

Table 1: On comparison of mean scores between males and females among the three groups using one way ANOVA shows statically significant difference in reduction of *S mutans* concentration between 1 and 3. (Tables 2 and 3)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p-value
						Lower Bound	Upper Bound		
Before	1.00	20	1.84	0.15	0.03	1.77	1.91	0.020	0.981
	2.00	20	1.84	0.14	0.03	1.77	1.91		
	3.00	20	1.83	0.25	0.05	1.72	1.94		
	Total	60	1.84	0.18	0.02	1.79	1.88		
After	1.00	20	1.56	0.17	0.04	1.48	1.63	147.805	0.001*
	2.00	20	1.49	0.14	0.03	1.43	1.55		
	3.00	20	0.89	0.09	0.02	0.84	0.93		
	Total	60	1.31	0.33	0.04	1.22	1.40		

Table 2: Comparison of mean scores in males among three groups using one way ANOVA

Male		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p-value
						Lower Bound	Upper Bound		
Before	Control	7	1.94	0.14	0.05	1.81	2.07	1.315	0.291
	Fluoridated Toothpaste	9	1.93	0.11	0.04	1.85	2.02		
	Probiotic Toothpaste	7	1.84	0.14	0.05	1.71	1.97		
	Total	23	1.91	0.13	0.03	1.85	1.97		
After	Control	7	1.70	0.13	0.05	1.58	1.82	105.117	0.001*
	Fluoridated Toothpaste	9	1.60	0.12	0.04	1.51	1.69		
	Probiotic Toothpaste	7	0.90	0.08	0.03	0.82	0.98		
	Total	23	1.42	0.37	0.08	1.26	1.58		

Table 3: Comparison of mean scores in females among three groups using one way ANOVA

	Female	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p-value
						Lower Bound	Upper Bound		
Before	Control	13	1.78	0.13	0.04	1.71	1.86	.279	0.758
	Fluoridated Toothpaste	11	1.76	0.11	0.03	1.69	1.84		
	Probiotic Toothpaste	13	1.82	0.29	0.08	1.65	2.00		
	Total	37	1.79	0.19	0.03	1.73	1.86		
After	Control	13	1.48	0.14	0.04	1.39	1.56	126.502	0.001*
	Fluoridated Toothpaste	11	1.40	0.06	0.02	1.36	1.44		
	Probiotic Toothpaste	13	0.88	0.09	0.03	0.82	0.93		
	Total	37	1.24	0.29	0.05	1.15	1.34		

3. Results

After one month application of non-fluoridated toothpaste (group 1), fluoridated toothpaste (group 2) and probiotic toothpaste (group 3), the level of *streptococcus mutans* significantly reduced in group 2 (fluoridated toothpaste) and group 3 (Probiotic toothpaste) but minimal reduction is shown in group 1. (non fluoridated toothpaste) as shown in Table 1.

4. Discussion

Over a span of time, we realize that esthetics in orthodontics is the ultimate challenge. Decalcifications in the form of white spot lesions appear frequently in orthodontic patients as small lines along the bracket periphery and in a few patients as large decalcifications with or without cavitations.

Enamel demineralization, or white spot lesions, a common esthetic hurdle in orthodontic treatment, is increased when a patient's oral hygiene is poor. It is mainly caused by the organic acids produced by cariogenic microorganisms in the oral cavity. The prevalence of white spot lesions was reported to be as high as 50% in some

patients.[2] The demineralization of enamel, a precursor of the carious lesion, appears opaque because of the decreased light-scattering ability of the decalcified enamel.

The difference found in white spot incidence on maxillary central and lateral incisors, whether banded or bonded, suggests that small tooth surface areas between the gingiva and the bracket are conducive to the retention of plaque and debris. This suggests the desirability of bracket placement that leaves adequate distance in the labiogingival area for more efficient hygiene.[12] In the anterior region, the lateral incisors are the most susceptible because of decreased salivary clearance and also less space between the bracket and gingiva. This creates less accessibility for oral hygiene techniques. White spot lesions in the anterior region are clearly visible and are a major esthetic concern.[18]

It was shown that the cariogenic *streptococci* have a very low binding affinity. Approximately 0.2–0.3% of the cells adhered to the metal brackets during the nine hours of incubation time.[19] This may be primarily because of the inherent low binding affinity of cariogenic *streptococci.* This is consistent with a previous study,[19] which showed that the proportion of *S. mutans* was smaller than the other *streptococci* and comprised only 0.5% of the dental plaque after 24 hours.

The adhesion of *S mutans* is promoted mainly by high-molecular weight mucin and slightly by acidic proline-rich proteins (acidic-PRPs) from saliva. The high-molecular weight mucin was not found, whereas the acidic-PRPs were detected from the salivary pellicle formed on the various brackets.[20,21]

White spot lesions have been prevented over the years using topical fluorides in the form of varnishes, dentifrices, and sealants. They reduce the number and size of white spot lesions. In orthodontics, continuous fluoride release has been developed with fluoride releasing elastomeric chains, glass ionomer cement, and composites for long treatment periods. Many other methods, including antimicrobial and antibiotic therapies, have been tried, but their efficiency lasts only as long as they are supplied at regular intervals.[5] These disadvantages have caused probiotics to be considered an efficient alternative that could be available in regular dietary supplements without causing major side effects.

The use of probiotics has taken giant leaps since the 20th century. Probiotics have been used in modifying the microbial flora of the stomach and intestines.[16] Probiotics can create a biofilm, acting as a protective lining for oral tissues against oral diseases by keeping the bacterial pathogens off oral tissues by filling the spaces where the pathogens would invade. Some probiotic species also secrete antimicrobial compounds called bacteriocins: eg, reuterin.,[22] The efficiency of probiotics can be improved by the use of prebiotics. The probiotic organisms, lactobacillus, streptococci, and bifidobacterium species, are genetically designed.

A study in which the effect of probiotic yogurt was evaluated on *S mutans* counts in the saliva of orthodontic patients showed that the number of subjects with high *S mutans* counts decreased from 63% to 21% after 2 weeks of consumption.[14] We chose to evaluate the plaque around orthodontic brackets because it would be a more accurate measure of the *S mutans* that cause white spot lesions.

Many studies have used saliva to determine the amounts of *S mutans* in the oral cavity.[7,14,15,23]

The major disadvantage of using saliva is that the *S mutans* in the saliva is a total count of the organisms in the oral cavity from previous carious lesions, the tongue, and other sites that harbor the organisms, and it is not specific to the tooth surface.[24]

An examination included location of incipient caries lesions, fillings, and crowns. *Mutans streptococci* were detected on 40% of all tooth surfaces. The frequency distribution of *mutans streptococci* and the level of colonization showed a decreasing gradient from molars to incisors for buccal, lingual, occlusal, and approximal surfaces. The location and number of approximal restorations were closely related to the colonization level of mutans streptococci except for second and third molars. Restored surfaces tended to be more colonized by mutans streptococci than sound surfaces, except for occlusal surfaces. A high prevalence of mutans streptococci was found in plaque samples from tooth-colored fillings, especially on buccal and lingual surfaces.[11,17] Since the anterior region has less salivary clearance, plaque accumulation around orthodontic brackets is a more specific region that harbors the *S mutans* and can be considered more reliable in regard to white spot lesions.

Since the anterior region has less salivary clearance, plaque accumulation around orthodontic brackets is a more specific region that harbors the *S mutans* and can be considered more reliable in regard to white spot lesions. An earlier study in orthodontic patients showed the efficiency of systemic consumption of probiotic yogurt for reducing the amount of *S mutans* in the saliva. Similar study by jose. et al. shows the efficacy of systemtic administration of probiotic curd and local administration of probiotic toothpaste.

Results of our study shown significant reduction in s mutans count around orthodontic brackets. This reduction can be attributed to the formation of a biofilm that prevents adhesion of pathogens, the competitive inhibition of pathogenic bacteria, and the antimicrobial agents produced by the bacteria.

5. Conclusion

The topical application of probiotic toothpaste caused significant decreases in the S mutans levels in the plaque around different orthodontic brackets of orthodontic patients. Although the difference between male and female is not that significant but a result shows that oral hygiene

of female patients was better than male patients. This was a short-term study, and a longer period of evaluation would establish the long-term advantages of probiotics and prebiotics in orthodontic patients and the relative merits of systemic vs. local probiotic therapy and application of probiotic toothpaste around different fixed and functional appliances.

6. Source of Funding

None.

7. Conflict of Interest

None.

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