

Evaluation of Effect of Probiotic Bacteria on the Level of Streptococcus Mutans in Plaque in Fixed Orthodontic Patients Using Polymerase Chain Reaction Technique - A Randomized Controlled Trial

Seema Grover¹, M.S Sidhu², Namrata Dogra^{3*}, Gowri Sankar⁴, Ashish Dabas⁵, Anudeep Kaur⁶

^{1,5}Professor, Department of Orthodontics,SGT University, Gurugram, Haryana, India

²Private Practitioner, Dr Sidhu's Dental Clinic, Vasant Kunj ,New Delhi

³Reader, Department of Orthodontics, SGT University, Gurugram, Haryana, India

⁴Professor, Department of Orthodontics, Narayana Dental College, Nellore,

⁶ Private Practitioner, Smile Dental Clinic, Rajasthan, India

ABSTRACT

The primary objective of this investigation was to assess effects of probiotic lozenges and an oral suspension containing probiotic bacteria on levels of Streptococcus mutans in plaque surrounding brackets in orthodontic patients using the Reverse Transcription -Polymerase Chain Reaction (RT-PCR) technique. This Randomized Controlled Trial was conducted on 45 patients of 14 to 25 years of age who required fixed orthodontic treatment. All participants were randomly assigned and divided into 3 groups of 15 each. Group I Patients were given probiotic lozenges, Group II Patients were given probiotic oral suspension, Group III Patients as control group and did not receive any probiotic treatment. The plaque samples were collected twice for all three groups, initially before beginning of the study (T0) and after 30 days (T1), and level of Streptococcus mutans in collected plaque samples was evaluated using the PCR technique. On intergroup comparison, mean reduction in Streptococcus mutans counts ($\log_{10}$ value) was significantly higher in probiotic lozenges group (3.4078); and probiotic oral suspension group (3.3326), than that of control group (0.2833). Probiotic lozenges and Probiotic oral suspension causes significant reduction in Streptococcus mutans levels in plaque of orthodontic patients with greater reduction in probiotic lozenges group.

KEY WORDS

Orthodontic Appliances, Fixed, Probiotic, Oral Suspension, Lozenges, Brackets, Streptococcus Mutans, Polymerase Chain Reaction.

INTRODUCTION

Many plaque retention sites are present in individuals with malocclusions due to irregularities of their teeth. So, in these individuals, maintenance of oral hygiene becomes difficult.¹ There is increased accumulation of plaque around the brackets and bands during fixed orthodontic therapy. Some studies have shown an increase in Streptococcus mutans and lactobacilli, in the plaque of orthodontic patients.²⁻⁵

One of the common iatrogenic effect of fixed orthodontic therapy is White spot lesions (WSL). The prevalence of white spot lesions arising during fixed orthodontic treatment ranges widely from 2 % to 96 %.⁶ Various methods have been used to prevent the formation of white spot lesions such as fluoride application and casein phosphopeptide amorphous calcium phosphate (CPACP).⁷⁻⁹

Probiotics are “live microbial food supplements that, when administered in adequate amounts, beneficially affect the host animal by improving its intestinal microbial balance”¹⁰ Traditionally, probiotics have been used for improving gut health, but several investigators have also suggested probiotics for oral health purposes.¹¹⁻¹⁴

Most common food containing probiotics is yogurt. Probiotics are useful in decreasing the level of Streptococcus mutans in the saliva.¹⁵⁻¹⁷ But literature is lacking in studies where the effect of probiotics has been evaluated in levels of Streptococcus mutans in orthodontic patients.

Bacteria can be detected in dental plaque by various methods. Some of them are bacterial culture such as phase-contrast microscopy, dark-field microscopy, immunologic assays, enzymatic assays, methods that detect bacterial DNA or RNA.¹⁸ The microbiologic culture technique is the most widely used method of detection of microbes. The drawbacks of this technique are that plaque samples must have bacteria that is viable and this process is time-consuming and laborious.¹⁸ Recently, there has been a strong emphasis on the PCR method for identifying pathogens because of its higher sensitivity and specificity compared with conventional methods. By this method, detection of small numbers of pathogens is possible using DNA fractions.¹⁹

Hence, the present study was undertaken to evaluate the effects of consumption of probiotic lozenges and an oral suspension containing probiotic bacteria on the levels of Streptococcus mutans in the plaque surrounding brackets in orthodontic patients using the RT-PCR technique.

MATERIAL AND METHOD

This Randomized Controlled Trial was conducted on 45 patients (30 females and 15 males), aged between 14 to 25 years who reported for fixed orthodontic treatment, to the Department of

Orthodontics. The study was approved by the ethical committee of the institution (SGTU / FDS / MDS / 21 / 1) on 17th December 2016.

Inclusion and Exclusion Criteria

The participants were selected under the following criteria - age between 14 to 25 years, patients having permanent dentition and good oral hygiene with twice brushing daily with fluoride toothpaste. But the participants - were excluded from the study under the following criteria - Patients suffering from systemic diseases or taking medication for the same, Patients on any other probiotic supplements during the study, regular xylitol chewing gum consumers, patients with systemic antibiotic or topical fluoride treatments within 4 weeks, patients with open or untreated caries lesions or gingival inflammation.

Methodology

It was a double-blind study and all the participants were randomly divided into 3 equal groups of 15 each. Randomization was conducted to computer generated allocation sequence of 1:1:1 to Group I, Group II and Group III. The researchers conducting the research were blind to the group allocation. Sample size was calculated using power analysis evaluation established by G* power , version 3.0.1. A sample size of 45 would yield 80% power to detect significant differences .Group I patients were given probiotic lozenges (Allianz Biosciences Private Ltd. India) which contain Streptococcus faecalis T-110TM (30 million), Clostridium butyricumTO-ATM (2 million), Bacillus mesentericusTO-ATM (1 million), Lactic acid bacillus (50 million). After the first plaque sample had been collected before bonding at baseline (T0) and continued for the 30 days (T1). Proper instructions were given to the patients to take two lozenges each in the morning and night and keep the lozenges in the mouth to be dissolved by itself. Group II patients were given probiotic oral suspension (Virchow Biotech Pvt. Ltd. India) which contains Bacillus clausii (2 x 10⁹ spores) after the first plaque sample had been collected before bonding at baseline (T0) and continued for 30 days (T1). The patients were instructed to take one vial in the morning and one vial at night.

Group III patients did not receive any probiotic treatment during the study, (control group).The patients were advised to brush two times daily with fluoridated toothpaste for 2 minutes in all three groups. The patients were instructed to avoid consumption of any food 30 min to 1 hour, before and after having taking the lozenges and oral suspension, and were instructed not to consume any avoid chewing gums or antibiotics during the course of the study. The patients were given a probiotic consumption chart to fill in the details when they consumed probiotic lozenges and probiotic oral suspension.

A sterilized scaler was used to take the first sample of plaque before bonding at baseline (T0) for all the three groups from the labial surface of maxillary lateral incisors using a 4-pass technique which was proposed by Pellegrini et al.⁵⁰ Second sample was collected after 30 days of bonding (T1). The banding and bonding was done using a 0.022-in slot bracket with MBT prescription (Ortho Organizer inc, CA USA). Plaque samples were carefully transferred to

individual vials containing Tris-EDTA buffer transport media. Then vials were designated with unique participant identification code, sealed, and dispatched to the microbiological lab at the Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgaum, Karnataka. Microbiological evaluation of the plaque samples included identification and relative quantification of *Streptococcus mutans* through the RT-PCR technique. The PCR values were obtained as the number of DNA copies / ml.

Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS, version 21, IBM). Student's unpaired t-test analysis was performed using IBM SPSS Version 24.0 (Armonk, NY: IBM Corp.). P-value <0.05 was considered statistically significant. The intragroup comparison of mean $\log_{10}$ values of *S mutans* counts was done using the Wilcoxon rank-sum test. Kruskal Wallis test & Mann Whitney U test was used to do intergroup comparison of mean $\log_{10}$ values of *S mutans* counts. 0.05 was the level of statistical significance.

RESULTS

Table 1 illustrates the intragroup comparison of mean *Streptococcus mutans* counts in Group I, Group II and Group III at baseline (T0) and after 30 days of bonding (T1) calculated using Wilcoxon signed pair rank-sum test. The results showed highly statistically significant ($p < 0.01$) difference in mean *Streptococcus mutans* counts after 30 days of bonding (T1) in Group I, statistically significant ($p < 0.05$) difference in mean *Streptococcus mutans* counts after 30 days of bonding (T1) in Group II and nonsignificant ($p > 0.05$) difference in mean *Streptococcus mutans* counts after 30 days of bonding (T1) in Group III.

	At base line (T0)		After 30 days (T1)		Mean Difference	P ^a Value
	Mean	Std. Deviation	Mean	Std. Deviation		
Group I	8.0591	1.10113	4.6513	0.81765	-3.4078	0.008**
Group II	8.7294	1.78682	5.3968	0.67510	-3.3326	0.012*
Group III	7.2769	1.75440	6.9937	1.97948	-0.2832	0.594 (NS)
Table 1. Mean change of <i>Streptococcus Mutans</i> count ($\log_{10}$ value) in Group I, Group II and Group III from T0 (baseline) to T1 (30 days post bonding).						
^a Wilcoxon signed pair rank sum test						
Not Significant - $p > 0.05$; Significant (*) $p < 0.05$; Very Highly Significant (***) $p < 0.001$						

Table 2 represents the comparison of mean *Streptococcus mutans* counts among Group I, Group II, and Group III after 30 days of bonding (T1) calculated using the Kruskal Wallis

test. Statistically significant difference ($p < 0.05$) was found in mean Streptococcus mutans counts among Group I, Group II, and Group III after 30 days of bonding (T1).

Group	N	Mean	Std. Deviation	95 % Confidence Interval for Mean		P ^b Value
				Lower Bound	Upper Bound	
Probiotic lozenge (Group I)	9	4.6513	0.81765	4.0228	5.2798	0.044*
Probiotic oral suspension (Group II)	9	5.3738	0.63524	4.8856	5.8621	
Control (Group III)	13	6.5555	2.11156	5.2795	7.8315	
Table 2. Comparison of mean Streptococcus Mutanscount (log₁₀ value) among Group I, Group II and Group III after 30 days of bonding (T1) using 95 % Confidence Interval						
^b Kruskal Wallis test Significant (*) p<0.05						

Table 3 represents the intergroup pairwise comparison of mean Streptococcus mutans counts between the three groups after 30 days of bonding (T1). The pair-wise differences were explored by post hoc Mann Whitney U test. The p-value found to be significantly higher ($p < 0.05$) in the control group (Group III) as compared to the probiotic lozenges group (Group I) which proves that there were higher Streptococcus mutans concentrations in the control group (Group III) than the probiotic lozenges group (Group I) after 30 days of the intervention period (TI). While no statistically significant differences ($p > 0.05$) were found between the Group I, Group II and; Group II, Group III.

Groups	Mean Difference	Post hoc Pairwise Comparison ^c
Group I - Group II	-0.7225	0.058 (NS)
Group I - Group III	-1.9042	0.03*
Group II - Group III	-1.1817	0.253 (NS)
Table 3. Inter-group comparison of mean Streptococcus Mutans count ($\log_{10}$ value) in Group I, Group II and Group III after 30 days of bonding (T1)		
^c Mann Whitney U test Not Significant - $p > 0.05$; Significant (*) $p < 0.05$		

Table 4 represents the comparison of reduction of mean in Streptococcus mutans counts among Group I, Group II, and Group III from baseline (T0) to 30 days post bonding (T1) done using Kruskal Wallis test. The results showed a statistically very highly significant difference (p

= 0.001) of mean reduction in Streptococcus mutans counts among Group I, Group II and Group III after 30 days of bonding (T1).

Group	N	Mean Difference (T1-T0)	Std. Deviation	95 % Confidence Interval for Mean		P ^b Value
				Lower Bound	Upper Bound	
Probiotic lozenge (Group I)	9	3.4078	0.51840	3.0094	3.8063	0.001***
Probiotic oral suspension (Group II)	8	3.3326	1.74522	1.8736	4.7917	
Control (Group III)	11	0.2833	1.48581	-.7149	1.2814	
Table 4. Comparison of reduction of mean Streptococcus Mutans count (log₁₀ value) among Group I, Group II and Group III from T0 (baseline) to T1 (30 days post bonding) using 95 % confidence interval						
^b Kruskal Wallis test Very Highly Significant (***) p<0.001						

Table 5 represents the intergroup comparison of mean reduction in Streptococcus mutans counts between Group I and Group II, Group I and Group III, Group II and Group III from baseline (T0) to 30 days after bonding (T1) done by Post hoc pairwise comparison using Mann Whitney U test. Mean reduction in Streptococcus mutans counts was significantly higher in the Group I; ($p < 0.001$) and Group II; ($p < 0.01$) than that of the Group III. It proves that there were higher Streptococcus mutans concentrations in the control group than the probiotic groups after 30 days of the intervention period.

Groups	Mean Difference	Post hoc pairwise comparison ^c
Group I - Group II	0.0752	0.501 (NS)
Group I - Group III	3.1245	<0.0001***
Group II - Group III	3.0493	0.002**
Table 5. Intergroup comparison of reduction of mean Streptococcus Mutans count ($\log_{10}$ value) in Group I, Group II and Group III from T0 (baseline) to T1 (30 days post bonding)		
^c Mann Whitney U test Not Significant - $p > 0.05$; Highly Significant (**) $p < 0.01$; Very Highly Significant (***) $p < 0.001$		

DISCUSSION

The risk of white spot lesions due to increased accumulation of bacterial plaque in orthodontic patients.^{20,21} This is due to the fact that dental plaque of orthodontic patients have increased retention of Streptococcus mutans and lactobacilli.²²

A multimodal approach was required for the control and treatment of dental caries, multifactorial disease of bacterial origin. Some probiotics containing foods such as cheese, and yogurt have been used in this regard.²³ Lactobacillus and Bifidobacterium are the most common strains which are found in the oral cavity. The probiotics help in neutralizing acidic conditions in the mouth, thus interfering with cariogenic bacteria. The probiotics produce a combination of local and systemic immune response and also act by defense mechanisms.²⁴ Probiotics fill the spaces which could be invaded by pathogens by developing a biofilm, thus protecting the oral tissue.²⁵

Usually prevalence of white spot lesions is maximum in maxillary lateral incisors on the facial tooth surfaces around the brackets, in the present study, a circum-bracket plaque collection technique was applied as suggested by Forsberg et al.²

Small amount of bacteria can easily be detected using Polymerase chain reaction (PCR). The maxillary lateral incisors were selected as the donor sites for microbial samples. It has been reported in previous studies that lateral incisors were found to have more plaque accumulation than other anterior teeth.²⁵ Therefore pooled sample from the maxillary right and left lateral incisors was collected for PCR microbial evaluation.

Intragroup comparison of mean Streptococci mutans counts showed a gradual decrease from baseline (T0) to 30 days intervention period (T1). There was a decrease in mean Streptococci mutans counts by 3.4078 which was highly significant ($p < 0.01$). These results are in correlation with the study done by Caglar et al.²⁶ They explained probiotic mechanism as host immune response which results in increased resistance to pathogens, change in the metabolic activity of host-microbiota at the specific location and production of antimicrobial substances and competition with pathogens for binding sites.

Intragroup comparison of mean Streptococci mutans counts showed a gradual decrease from baseline (T0) to 30 days intervention period (T1). There was a decrease in mean Streptococcus mutans counts by 3.3326 which was significant. These results are in accordance with the study done by Cildir et al.¹⁶ Intergroup comparison showed significantly higher ($p < 0.05$) Streptococcus mutans concentrations in Group III (Control group) than Group I (Probiotic lozenges group) after 30 days (T1) of bonding. This proves that the use of Probiotic lozenges efficiently reduced the Streptococcus mutans levels in plaque around orthodontic brackets. There were no statistically significant differences between Group I (Probiotic lozenges group), Group II (Probiotic oral suspension group), and Group III (Control group) ($p > 0.05$). It could be explained by the fact that the lozenges have direct contact with the oral mucosa when compared with the oral suspension. The findings of the present study are supported by the study done by Caglar et al, where they found that probiotic lozenge produced a statistically significant reduction in Streptococcus mutans to the placebo tablet when compared with the probiotic straw to the placebo straw ($p < 0.05$).²⁶

Significant reduction ($p < 0.001$) of Streptococci mutans counts was found between baseline and 30th day in Group I (Probiotic lozenges group), Group II (Probiotic oral suspension group), and Group III (Control group). The reduction in Streptococcus mutans counts was significantly higher in Group I (Probiotic lozenges group) and Group II (Probiotic oral suspension group) than that of Group III (Control group). It proves that there were higher Streptococcus mutans

concentrations in Group III (Control group) than the Probiotic groups after 30 days of the intervention period. This shows that the use of Probiotic lozenges and Probiotic oral suspension are effective means to reduce the *Streptococcus mutans* levels in plaque around orthodontic brackets.

CONCLUSION

Probiotic lozenges and Probiotic oral suspension causes a significant reduction in *Streptococcus mutans* counts in the plaque of patients undergoing orthodontic therapy with a greater reduction in probiotic lozenges group. Based on the observed results, it can be concluded that probiotic lozenge is more effective than oral suspension and daily short-term ingestion of probiotics could reduce the levels of *Streptococcus mutans* in plaque surrounding orthodontic brackets. This indicates that probiotics may be used as adjuncts, for the prevention of dental caries especially as a part of dietary modification in orthodontic patients at varying risk for dental caries.

REFERENCES

1. Ogaard B. White Spot Lesions During Orthodontic Treatment: Mechanisms and Fluoride Preventive Aspects. *Semin Orthod* 2008;14:183-193.
2. Forsberg CM, Brattström V, Malmberg E, Nord CE. Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of *Streptococcus mutans* and *Lactobacilli*. *Eur J Orthod* 1991;13:416-20.
3. Rosenbloom RG, Tinanoff N. Salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. *Am J Orthod Dentofacial Orthop* 1991;100:35-7.
4. Huser MC, Baehni PC, Lang R. Effects of orthodontic bands on microbiologic and clinical parameters. *Am J Orthod Dentofacial Orthop* 1990;97:213-8.
5. Fournier A, Payant L, Bouclin R. Adherence of *Streptococcus mutans* to orthodontic brackets. *Am J Orthod Dentofacial Orthop* 1998;114:414-7.
6. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod* 1982;81:93-8.
7. Bishara SE, Ostby AW. White spot lesions: formation, prevention and treatment. *Semin Orthod* 2008;14:174-82.
8. Willmot D. White spot lesions after orthodontic treatment. *Semin Orthod* 2008;14:209-19.
9. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J* 2006;14:284-9.
10. Anderson MH, Shi W. A probiotic approach to caries management. *Pediatr Dent* 2006;28:151-3.
11. Reeves J. Probiotics—a new approach to oral health. July 2008. Available at: <http://www.dentistry.co.uk/articles/probiotics-newapproach-oral-health>. Accessed December, 2012.
12. Reeves J. Oral health and the renal patient. *Dent Nurs* 2008;4:618-21.

13. Hillman JD, McDonell E, Hillman CH, Zahradnik RT, Soni MG. Safety assessment of ProBiora3, a probiotic mouthwash: subchronic toxicity study in rats. *Int J Toxicol*2009;28:357-67.
14. Caglar E, Kusu OO, Cildir SK, Kuvvetli SS, Sandalli N. A probiotic lozenge administered medical device and its effects on salivary streptococci and lactobacilli. *Int J Pediatr Dent* 2008;18:35-9.
15. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic lactobacillus reuteri. *Swed Dent J* 2006;30:55-60.
16. Cildir SK, Germac D, Sandalli N, Ozdemir FI, Arun T, Twetman S, Caglar E. Reduction of salivary mutans streptococci on orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. *Eur J Orthod*2009;31:407-11.
17. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. *Acta OdontolScand*2005;63:317-20.
18. Listgarten MA. Microbiological testing in the diagnosis of periodontal disease. *J Periodontol*1992;63:332-7.
19. Igarashi T, Yamamoto A, Goto N. PCR for detection and identification of Streptococcus sobrinus. *J Med Microbiol* 2000;49:1069-74.
20. Mizrahi E. Surface distribution of enamel opacities following orthodontic treatment. *Am J Orthod*1983;84:323-31.
21. O'Reilly MM, Featherstone JDB. Demineralization and remineralization around orthodontic appliances: An in vivo study. *Am J OrthodDentofacOrthop*1987;92:33-40.
22. Corbett JA, Brown LR, Keene HJ, Horton IM. Comparison of Streptococcus mutans concentrations in non-banded and banded orthodontic patients. *J Dent Res* 1981;60:1936-42.
23. Sudhir R, Praveen P, Venkataraghavan K. Assessment of the effect of probiotic curd consumption on salivary pH and streptococcus mutans counts. *Nigeria Med J* 2012;53(3):135-39.
24. Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' possible role on oral health. *Oral Dis* 2005;11:131-7.
25. Chaturvedi S, Jain U, Prakash A, Sharma A, Shukla C, Chhajed R. Efficacy of probiotic lozenges to reduce Streptococcus mutans in plaque around orthodontic brackets. *J Indian Orthod Soc* 2016;50:222-7.
26. Caglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacillus levels after ingestion of the probiotic bacterium lactobacillus reuteri ATCC 55730 by straws or tablets. *Acta OdontolScand*2006;64:314-8.