A Comparative Study of Salivary Streptococcus Mutans Count Before and After Probiotic Intervention in Multi-Bracket Fixed Orthodontic Patient Group-An In-Vivo Study

Jayanti M. Hegde[1], Deepthi Shiri[2], Kiran Kumar Hc[3]

[1]Lecturer, Bapuji Dental College And Hospital, Karnataka
[2]Professor, Bapuji Dental College And Hospital, Karnataka
[3]Professor & Head Of The Dept, Bapuji Dental College And Hospital, Karnataka

ABSTRACT

AIMS AND OBJECTIVES: The study aims to investigate the effect of regular probiotic toothpaste on salivary Streptococcus mutans in patients with fixed orthodontic appliances.

METHOD: 21 Patients prescribed fixed orthodontic therapy were selected for the study. Unstimulated saliva was collected from each patient in the study at four points of time: 1) T_0 - Before bonding stainless-steel multi-brackets. 2) T_1 -12 weeks after fixed orthodontic treatment (Colgate ™ toothpaste). 3) T_2 - 12 weeks after probiotic toothpaste intervention. 4) T_3 - 12 weeks after Colgate ™ toothpaste usage. At each time point, salivary Streptococcus mutans colonies and salivary pH were noted.

RESULTS: The findings showed an increase in the number of salivary Streptococcus mutans count from T_0-T_1, T_1-T_2, & T_2-T_3. There was a decrease in the salivary pH from T_0-T_3. There was a significant negative correlation between the number of salivary Streptococcus mutans colonies count and pH at all 4 time points. The higher the colony count, the lower the pH. Further, the correlation was strongest at T3, followed by T2, T1, and T0.

CONCLUSION: The usage of probiotics had a significant effect neither on Streptococcus mutans nor on pH. Further, probiotics failed to maintain their impact following the stopping of their usage.

KEYWORDS:
Probiotics, Salivary Streptococcus mutans, Fixed Orthodontic Patients.

INTRODUCTION

Fixed orthodontic treatment creates secure places for food retention and hence an ecological niche due to braces, bands, and arch wires. This poses challenges to oral hygiene by hindering cleaning procedures and altering salivary characteristics and microbial counts. The difficulty in maintaining oral hygiene creates an ecological niche that promotes the growth of microorganisms, leading to enamel demineralization. This occurs as dietary carbohydrates are fermented by bacteria like Streptococcus mutans and Lactobacillus, lowering the pH of dental plaque.[1] This leads to the formation of white spot lesions which is a serious concern to orthodontists and patients. It has been documented that the average prevalence of white spot lesions in patients undergoing orthodontic treatment is between 4.9% to 84%. [2] The number of Streptococcus mutans count can increase by up-to 5 fold during the orthodontic treatment, which has been identified as the major microorganism in dental plaque causing demineralization and subsequent tooth decay. [3]

Different techniques have been suggested to prevent or reverse enamel demineralization. Few of them being proved to be useful are fluoride delivery systems, casein phosphopeptide amorphous calcium phosphate, enamel surface attenuation with an argon laser, continuous fluoride release from fluoride-containing sealants, elastomeric chains, primers, and adhesives in bonding brackets. However, the application of fluoride has the disadvantage of requiring frequent follow-ups to the dentist. [2]

The Ukrainian-born biologist and Nobel laureate Elie Metchnikoff found out in 1907 that consumption of Bulgarian yogurt (which contains lactic acid bacteria) was beneficial. Probiotics are "live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance."[2] Bacterial therapy or replacement treatment is an alternative way of fighting infections by using harmless bacteria instead of pathogenic microorganisms.[1] Lactic acid bacteria and bifidobacteria are commonly used probiotics, although certain yeasts and bacilli are also utilized. These probiotics adhere well to tissues and competitively inhibit pathogenic bacteria without affecting beneficial bacteria. Research indicates that once pathogenic species are replaced, their reintroduction is hindered. [2]

Since the 20th century, the use of probiotics has increased exponentially. By forming a biofilm on oral tissues, probiotics act as a protective layer against oral diseases by keeping bacteria from invading these tissues by filling the spaces where they would normally invade. [4]

There are limited studies demonstrating the effect of probiotic products in orthodontic patients. Therefore, in this study, we aim to determine the effect of regular probiotic toothpaste usage on salivary Streptococcus mutans colonization in patients receiving fixed orthodontic treatment.

METHODOLOGY

Patients who were prescribed fixed orthodontic therapy using stainless steel multi-bracket appliance at a Dental College and Hospital.

SAMPLE SIZE DETERMINATION- sample size was 16 but considering dropouts it was rounded up to 20

METHOD OF COLLECTION OF DATA:

Criteria for selection of sample:

Inclusion criteria:

- Patients who were prescribed fixed orthodontic therapy using stainless steel multi-bracket appliance.
- Good general health.
- Age = 12-25 years
- Fewer than 5 decayed, missing, or filled teeth.

Exclusion criteria:

- Patients treated with ceramic brackets, removable appliances and aligners.
- Patients having any systemic disease.

Materials required:

- Probiotic toothpaste(Purexa^{TM})
- Colgate^{TM} toothpaste
- Orthodontic toothbrush for the patients (colgate^{TM})
- pH strips
- Specimen container
- Mitis- Salivarius agar media, Potassium tellurite, and Bacitracin. (Hi-media^{TM})

Method of study:

A detailed case history was taken for each patient. Patients who met inclusion and exclusion criteria were given information about the study and informed consent was obtained from patients willing to participate in the study. Before the beginning of the fixed orthodontic treatment, all patients were given oral hygiene instructions and advised to brush twice daily. To ensure standardization, all the patients were provided with an orthodontic toothbrush and a toothpaste and asked to brush twice daily, morning and evening by dispensing peanut-sized quantity of toothpaste each time, after orthodontic bracket bonding for 12 weeks. Following this, all the patients were provided with probiotic toothpaste (Purexa contains Bacillus coagulans) and asked to brush twice daily, morning and evening by dispensing 1-inch strip of toothpaste each time for the next 12 weeks. Subsequently, all the patients were provided with Colgate toothpaste and asked to brush twice daily by dispensing a peanut-sized quantity of toothpaste each time, morning and evening for the next 12 weeks. Orthodontic toothbrushes were replaced every 3 months. Toothpastes which were provided to the patients were wrapped for blinding purpose & hence reducing the bias [Figure 1 & 2]. All patients were instructed to report to the Department of Orthodontics and Dentofacial Orthopedics, immediately, if any symptoms of allergy to the toothpaste develop. During the study, all patients were asked to stop using their regular toothbrush and toothpaste. To eliminate bias, all toothpaste tubes that were provided to patients were masked.

Unstimulated saliva was collected from each patient at four points of time:

- Flowchart depicting the timings of collection of saliva specimen:

T_0- Before bonding of stainless-steel multi-brackets

↓

Immediately following which stainless-steel multi-brackets will be bonded

12 weeks — Colgate ™

T1

12 weeks — probiotic intervention

T2

12 weeks — Colgate ™

T3

All the patients in the study were informed not to eat or drink anything at-least 1-2 hours before collection of saliva. Unstimulated saliva was collected in sterilized specimen container [Figure 3]. Salivary pH was obtained with pH strips at all 4 point of time mentioned above. Mitis- Salivarius agar media with Potassium tellurite and Bacitracin was used to culture Streptococcus mutans at 37°C for 48 hours in incubating machine available in Department of Oral pathology and Microbiology, which was followed by counting the Streptococcus mutans colonies [Figure 4 & 5].

Fig 1- Blinded Probiotic toothpaste

Fig 2- Blinded Colgate toothpaste

Fig 3- Unstimulated saliva was collected in sterilized specimen container

Fig 4- Streptococcus mutans colony on agar media

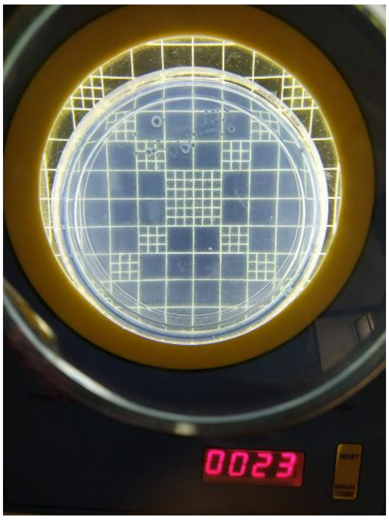


Fig 5- Digital colony counter

All the procedures were performed in compliance with relevant laws and institutional guidelines and have been aproved by the appropriate institutional review board. Informed consent was also obtained prior to the study from each patient. The privacy rights of all patients were kept confidential.

RESULTS

The present study determines the effect of regular probiotic toothpaste usage on salivary Streptococcus mutans colonies and pH of saliva in patients receiving fixed orthodontic treatment.

The present study consisted of one set of study group who are undertaking fixed orthodontic treatment and were evaluated for Streptococcus mutans colonies and pH at 4 different time points over a period of 9 months. Salivary specimen were collected at time points as follows-

T_0- Before bonding of stainless-steel multi-bracket

T_1- 12 weeks after stainless-steel multi-brackets were bonded (Colgate ^{TM} tooth paste)

T_2- 12 weeks after probiotic toothpaste intervention

T_3- 12 weeks after Colgate ^{TM} toothpaste usage.

pH & the colonies were counted & noted.

Analysis summary

The data were analyzed using IBM SPSS version 25. The data were skewed and hence non-parametric test- Wilcoxon-Sign rank- was used to compare the pH and bacterial counts at different time points. Pearson's correlation analysis was used to correlate the pH and bacterial count. A "p" value of <.05 was considered significant for all analyses.

Streptococcus mutans colonies (Table 1-5, Graph 1):

- In the present study group, there was a statistically significant increase in Streptococcus mutans colonies from T0 to T3 at every time point with a "p" value of 0.001 (<0.05). (Table i)
- From T_0-T_1 there was a statistically significant increase in Streptococcus mutans colonies with a "p" value of 0.015 (<0.05). (Table ii)
- From T_1-T_2 there was a statistically significant increase in Streptococcus mutans colonies with a "p" value of 0.002 (<0.05). (Table iii)
- From T_2-T_3 there was a statistically significant increase in Streptococcus mutans colonies with a "p" value of 0.001 (<0.05). (Table iv)
- There was a statistically significant increase in Streptococcus mutans colonies at T0 and T3 with a "p" value of 0.006 (<0.05). (Table v)

Table 1: Comparison of Streptococcus mutans colonies from T0 to T3 using Friedman's ANOVA.

Time	Median	Chi square	P value
T0	0	17.56	.001
T1	5		
T2	11		
T3	17		

*p<0.05 statistically significant p>0.05 Non significant

Table 2: Comparison of Streptococcus mutans colonies at T0 and T1.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T0	0	Negative	5.90	29.50	Z= -2.442
T1	5	Positive	10.88	141.50	P = .015

*p<0.05 statistically significant p>0.05 Non significant

Table 3: Comparison of Streptococcus mutans colonies at T1 and T2.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T1	5	Negative	2.00	2.00	Z= -3.171
T2	11	Positive	7.92	103.00	P = .002

*p<0.05 statistically significant p>0.05 Non significant

Table 4: Comparison of Streptococcus mutans colonies at T2 and T3.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T2	11	Negative	1.50	1.50	Z= -3.204
T3	17	Positive	7.96	103.50	P = .001

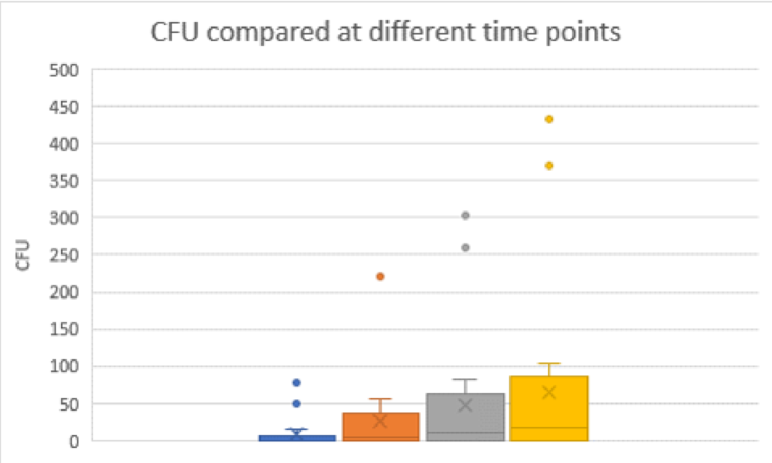
*p<0.05 statistically significant p>0.05 Non significant

Table 5: Comparison of Streptococcus mutans colonies at T0 and T3.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T0	0	Negative	4.60	23	Z= -2.72
T3	17	Positive	11.38	148	P = .006

*p<0.05 statistically significant p>0.05 Non significant

Graph 1: Comparison of Streptococcus mutans colonies (CFU- colony forming unit) from T0 to T3



Salivary pH at four different time points (Table 6-10, Graph 2)

- In the present study group, there was a statistically significant decrease in salivary pH from T0 to T3 at every time point with "p" value 0.035 (<0.05). (Table vi)
- From T_0-T_1 there was no statistically significant decrease in salivary pH with a "p" value of 0.083(>0.05). (Table vii)
- From T_1-T_2 there was no statistically significant decrease in salivary pH with a "p" value of 0.414(>0.05).v(Table viii)
- From T_2-T_3 there was no statistically significant decrease in salivary pH with a "p" value of 0.157 (>0.05). (Table ix)
- There was a statistically significant decrease in salivary pH at T0 & T3 at every time point with a"p" value of 0.04 (<0.05). (Table x)

Table 6: Comparison of salivary pH from T0 to T3 using Friedman's ANOVA.

Time	Median	Chi square	P value
T0	7	8.58	.035
T1	6		
T2	6		
T3	6		

*p<0.05 statistically significant p>0.05 Non significant

Table 7: Comparison of salivary pH at T0 and T1.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T0	7	Negative	6.50	58.50	Z= -1.732
T1	6	Positive	6.50	19.50	P = .083

*p<0.05 statistically significant p>0.05 Non significant

Table 8: Comparison of salivary pH at T1 and T2.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T1	6	Negative	2.25	4.50	Z= -.816
T2	6	Positive	1.50	1.50	P = .414

*p<0.05 statistically significant p>0.05 Non significant

Table 9: Comparison of salivary pH at T2 and T3.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T2	6	Negative	1.50	3.00	Z= -1.414
T3	6	Positive	.00	.00	P = .157

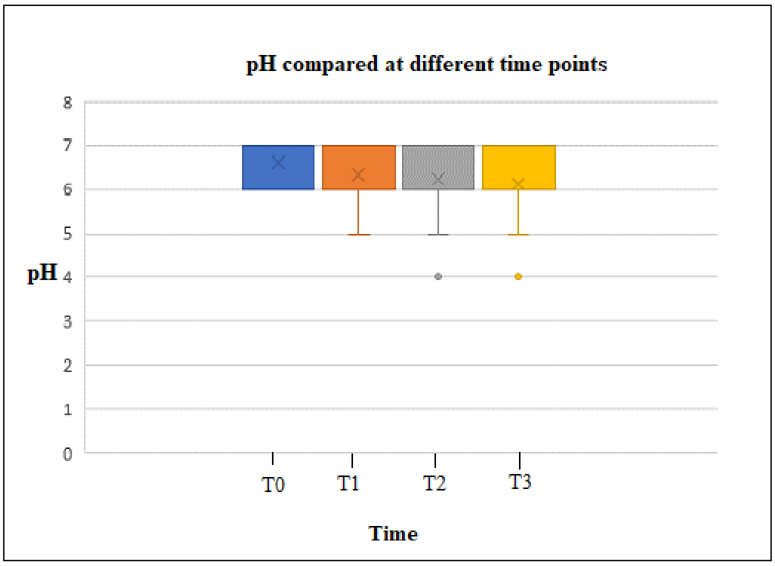
*p<0.05 statistically significant p>0.05 Non significant

Table 10: Comparison of salivary pH at T0 and T3.

Time	Median	Rank	Mean rank	Sum of ranks	Z (p value)
T0	7	Negative	8.55	94	Z= -2.05
T3	6	Positive	6.50	26	P = .04

*p<0.05 statistically significant p>0.05 Non significant

Graph 2: Comparison of salivary pH from T0 to T3



Correlation of Streptococcus mutans colonies with salivary pH at four different time points (Table 11, Graph 3-6)

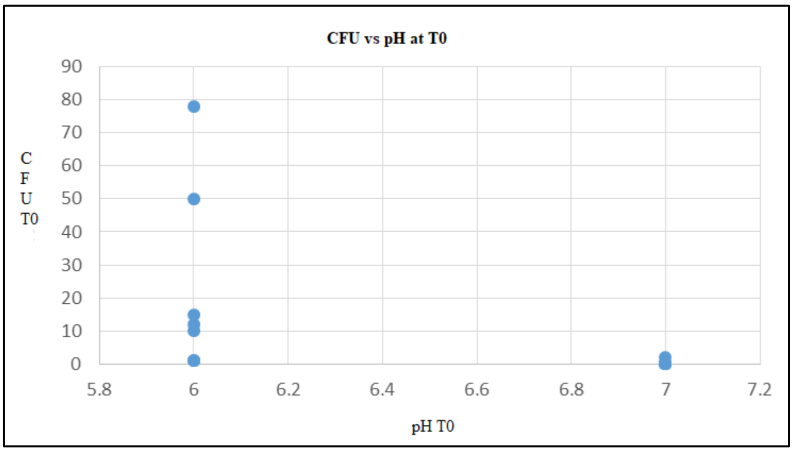
- In the present study group, there was a significant negative correlation between the Streptococcus mutans colonies count and pH at all 4-time points- the higher the colony count, the lower the pH. Further, the correlation was strongest at T3, followed by T2, T1 and T0.

Table 11: Correlation of Streptococcus mutans colonies with salivary pH at different time points presented through Pearson's correlation coefficient.

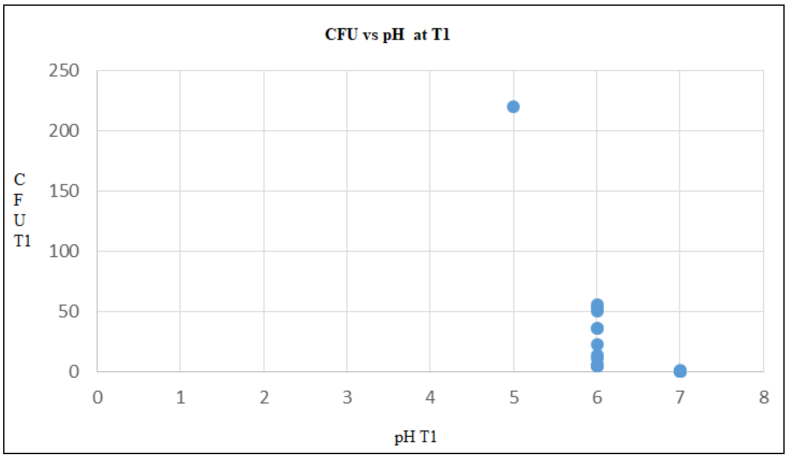
Time 1	r value	P value
T0	-.529	.014
T1	-.698	<.001
T2	-.859	<.001
T3	-.887	<.001

*p<0.05 statistically significant p>0.05 Non significant

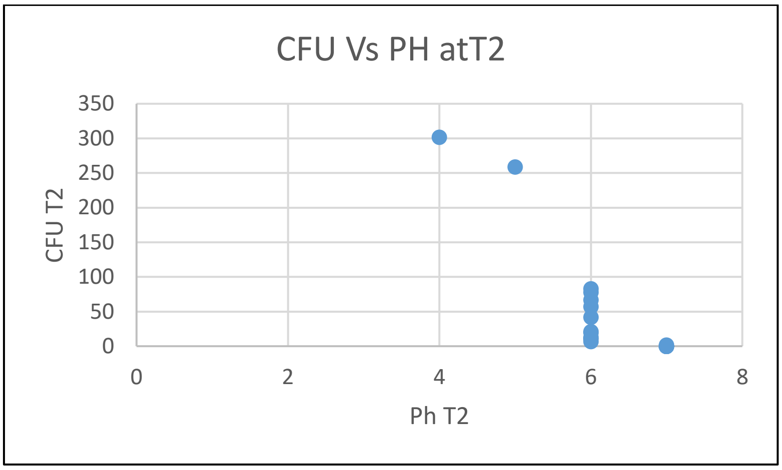
Graph 3: Correlation of Streptococcus mutans colonies with salivary pH at T0



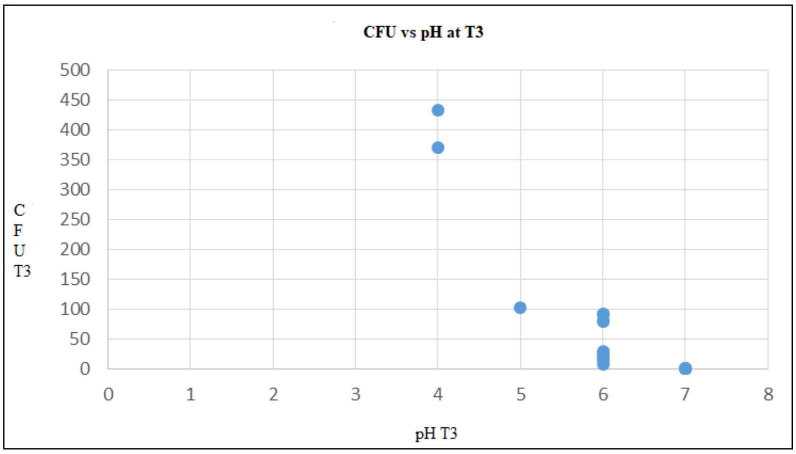
Graph 4: Correlation of Streptococcus mutans colonies with salivary pH at T1



Graph 5: Correlation of Streptococcus mutans colonies with salivary pH at T2



Graph 6: Correlation of Streptococcus mutans colonies with salivary pH at T3



DISCUSSION:

Effect of Probiotics on Streptococcus mutans

Elie Metchnikoff, a Nobel laureate and biologist from Ukraine, discovered in 1907 that Bulgarian yogurt, which contains lactic acid bacteria, was beneficial to health. [5] Probiotics are "live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance". [2]Hillman and colleagues concluded in their study that probiotics genetically modified Streptococcus mutans organism, so that, it no longer produces acid while competing aggressively for the ecologic niche where the wild-type Streptococcus mutans were found. This not only puts an end to the disease process but also prevents re-infection. [6]

Probiotics have been shown to reduce the risk of tooth decay, halitosis, and periodontal disease. Probiotics must adhere to the dental tissues and become part of the biofilm to create a cariostatic effect and fight the bacteria that cause tooth decay.[1] Hence we chose probiotic toothpaste in our study to find its effect on Streptococcus mutans.

Working mechanism of probiotics

To date, several working mechanisms of probiotics have been proposed, i.e., secretion of various antimicrobial substances such as organic acids, hydrogen peroxide, bacteriocins and biosurfactants. These substances fight against Streptococcus mutans strains to replace the naturally occurring cariogenic characteristics in them. In addition, they compete with pathogenic agents for adhesion sites on the mucosa. Probiotics can also change the surrounding environment by modulating the pH and/ or the oxidation−reduction potential, which can compromise the ability of pathogens to become established. A combination of probiotic strains is often used to increase these beneficial effects. [7]

The mechanism of probiotic action in the oral cavity is not well understood, but commonly explained by a combination of local and systemic immune response as well as non-immunologic defense mechanisms. The principal health promoting effects are ascribed to enhancement of mucosal immune defense and macrophage activity as well as elevations of the numbers of killer cells, T-cells, and interferon. [8- 10]

Each strain of probiotics has its own unique mechanisms of preventing dental plaque buildup and caries. These mechanisms are the result of a combination of competing actions. Buffering the saliva, production of bacteriocin and enzymes (dextranase, mutanase, and urease), the capacity to compete for the adhesion and colonization on tooth surfaces are other possible mechanisms behind the beneficial effect of probiotics. [11]

In the present study, the probiotic intervention started 3 months after the insertion of the appliances to avoid the confounding effect of an immediate decrease or increase in bacterial counts that may take place at appliance insertion. [10] In a study done by Scheie et al., he found out that several weeks were needed to create local conditions favorable for the multiplication of Streptococcus mutans. He found that banding the tooth covered the surfaces that were ecological niche for microbial organisms and hence, there was an immediate decrease in the Streptococcus mutans count. [01,12] There might be immediate increase in oral microorganisms following bonding of multi-brackets as patients' needs time to learn oral hygiene procedures with brackets and wire in the mouth. To eliminate these confounding factors, in the present study we decided to start probiotic (Purexa ™ toothpaste) therapy after 12 weeks into the multi-bracket fixed appliance. At the end of 12 weeks into probiotic therapy, saliva samples were collected, following which salivary pH and number of Streptococcus mutans were noted. In the results, we found that there was a statistically significant increase in the number of Streptococcus mutans colonies. This is following the study done by Montalto et al who reported an increased amount of Streptococcus mutans level. In this study, to assess systemic effects, patients were administered with Lactobacillus in fluid form and in capsules. They concluded that there was no reduction of Streptococcus mutans level following probiotic intervention. [13]

The present study is following the study done by Pinto GS et al who reported that ingestion of yogurt containing the probiotic B. animalis subsp. lactis DN-173010 did not reduce the counts of Streptococcus mutans, lactobacilli, and total microorganisms in saliva or dental plaque. [14]

Meurman, Nase et al, 2001; Ahola et al., 2002; Caglar et al., 2006; Teughels et al., 2008 stated that although dairy foods are considered useful vehicles for probiotic delivery, the best way of administration has yet to be identified. [08,15]

In a study done by Jose JE, Padmanabhan S, Chitharanjan AB in 2013, they concluded that the consumption of probiotic curd and the use of probiotic toothpaste cause a significant decrease in the Streptococcus mutans levels in the plaque around brackets in orthodontic patients. Although probiotic toothpaste was more effective than systemic consumption, this was not statistically significant.[2]

Simón-Soro and Mira stated that a wide array of microorganisms that produce organic acids and induce the decrease of salivary pH and dental plaque are associated in demineralization of enamel along with Streptococcus mutans. For this reason, we decided to examine salivary pH along with counting colonies of Streptococcus mutans, as an indirect measurement to determine the effect of the probiotic on the control of the cariogenic bacteria present in saliva. Unlike Lin et al. who found an increase in the minimum pH of the bioflm, in our study, the pH of saliva decreased continuously from the beginning till the end of the study. [15]

The result in the current study can be attributed to uncooperative patients who did not follow oral hygiene instructions and the amount of toothpaste to be used each time during brushing in spite of oral hygiene maintenance demonstration and strict warning to follow it. Cooperation towards maintenance of oral hygiene from patients during orthodontic treatment is utmost important in preventing enamel demineralization.

The results in our study also emphasize the importance of selecting an appropriate probiotic not only for its beneficial characteristics but also for its lack of cariogenic properties such as acid production. [11] However, all probiotics do not have the same efficacy. It is important that the potential probiotic strains are well characterized prior to use. A combination of strains can enhance adherence in a synergistic manner. Sookhee et al reported that the antimicrobial potentials of the bacteria were affected by several factors, such as pH, catalase, proteolytic enzymes and temperature. [15]

To evaluate the post-usage effects of probiotics, we provided (Colgate) toothpaste to all patients for 12 weeks. Though it was not statistically significant, results showed effects of probiotics were maintained only in the patients those who had less number of Streptococcus mutans from the beginning of the study. This could be attributed to the patients who maintained good oral hygiene had less number of Streptococcus mutans colonies and moderate pH level irrespective of the type of toothpaste used. Results in our study showed that probiotic toothpaste failed to reduce the number of Streptococcus mutans in the patients who had high colonies of the same in the beginning of the study. pH decreased further even after treatment with probiotic toothpaste which was statistically not significant. This was in contradiction to Glavina et al, who reported that salivary buffering capacity increased after 30 days of consuming yogurt containing L rhamnosus GG. [1]

Correlation between Streptococcus mutans and pH

In the present study, there was a significant negative correlation between Streptococcus mutans colony count and pH at all 4 time points-higher the colony count, lower was the pH. Further, the correlation was strongest at T3, followed by T2, T1 and T0.

CONCLUSION:

The present study determines the effect of regular probiotic toothpaste usage on salivary Streptococcus mutans colonies and salivary pH in patients receiving fixed orthodontic treatment.

1. There was a statistically significant increase in the colonies of salivary Streptococcus mutans following 12 weeks into multi-bracket fixed appliance treatment (Colgate toothpaste).
2. There was a statistically significant increase in the colonies of salivary Streptococcus mutans following 12 weeks into probiotic therapy. Hence, it was concluded that probiotics had nil effect on salivary Streptococcus mutans.
3. There was a statistically significant increase in the colonies of salivary Streptococcus mutans following 12 weeks into Colgate ™ toothpaste after probiotic therapy. Hence, it was concluded that probiotics were inefficient in maintaining its effect following cessation of its usage.
4. There was a reduction in salivary pH following 12 weeks into multi-bracket fixed appliance treatment but it was not statistically significant.
5. There was a reduction in salivary pH following 12 weeks into probiotic therapy but it was not statistically significant.
6. There was a reduction in salivary pH following 12 weeks into Colgate ™ toothpaste after probiotic therapy but it was not statistically significant.
7. There was a statistically significant negative correlation between the CFU count and pH at all 4 time points- the higher the colony count, the lower the pH. Further, the correlation was strongest at T3, followed by T2, T1, and T0.

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