Original Article

Analysis Of Streptococcus Mutans Level With The Use Of Different Mouth Rinses During Orthodontic Treatment

Dhruv Ahuja[1*], Prajwal Shetty[2], Shobha Giri[3], Azhar Mohammed[4],Vinayak Kamath[5], MS Ravi[6], Veena Shetty[7]

[1*] Department of Orthodontics and Dentofacial Orthopaedics, Manipal College of Dental Sciences Mangalore (Manipal Academy of Higher Education-Manipal) Karnataka, India, Email:- dhruvahuja96@gmail.com
[2] Additional Professor, Department of Orthodontics and Dentofacial Orthopaedics, AB Shetty Memorial Institute of Dental Sciences, NITTE (Deemed to be University) Mangalore, India, Email:- drprajwalshetty@gmail.com
[3] PhD, Department of Microbiology, KS Hegde Medical Academy, NITTE (Deemed to be University) Mangalore, India Email:- shobhasmc@yahoo.com
[4]Reader, Department of Orthodontics and Dentofacial Orthopaedics, AB Shetty Memorial Institute of Dental Sciences, NITTE(Deemed to be University)Mangalore, India, Email:- dr.azhar.mohd@gmail.com
[5] Lecturer, Department of Public Health Dentistry, Goa Dental College and Hospital, Goa, India, Email:- b_vk2002@yahoo.com
[6] Head of Department & Professor, Department of Orthodontics and Dentofacial Orthopaedics, AB Shetty Memorial Institute of Dental Sciences, NITTE(Deemed to be University)Mangalore, India, Email:- drmsravi@gmail.com
[7] Professor, Department of Microbiology, KS Hegde Medical Academy, NITTE (Deemed to be University) Mangalore, India, Email:- vndshetty@yahoo.co.in

***Corresponding Author**: - Dhruv Ahuja

* Department of Orthodontics and Dentofacial Orthopaedics, Manipal College of Dental Sciences Mangalore (Manipal Academy of Higher Education-Manipal) Karnataka, India, Email:- dhruvahuja96@gmail.com
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Abstract

Background:- Proper oral hygiene measures and techniques are always very crucial during Orthodontic treatment with fixed appliances. Orthodontic appliances create an oral environment such that there is an increase in plaque and debris attachment to the enamel surfaces which are difficult to clean and provide a source for bacterial accumulation, amongst which Streptococcus mutans play a major role in causing dental caries, gum diseases, decalcification and or staining of teeth. As a preventive measure, the use of mouth rinses is one of the commonly used methods to maintain oral hygiene during Orthodontic treatment. Hence this study is planned to analyse efficacy of 5 different mouth rinses in controlling the level of Streptococcus mutans, which is the most common causative factor for causing the dental decay.

Materials And Methods:- The study includes 30 orthodontic patients, classified into 6 groups, each group consisting of 5 male patients of age group 18-30 years were assessed for Streptococcus mutans level with the use of different mouth rinses.

Results:- The findings of this study showed that there is no statistically significant difference between the various groups, though chlorhexidine initially showed reduction in the levels of Streptococcus mutans level, followed by amine fluoride and essential oil based mouth rinses have reduced the levels of Streptococcus mutans at different time intervals.

Conclusion:- Mouth rinses are effective in reducing the Streptococcus mutans level during the treatment and can be used as preventive measure during orthodontic treatment.

Keywords:- Streptococcus mutans, mouth rinse, orthodontic appliances

I. INTRODUCTION

Orthodontic treatment with fixed appliances play a significant role in developing microbial plaque which causes enamel demineralisation. There is increase in number of carious lesion predominantly in the sites adjacent to brackets,[1] there are high chances of increase in *Streptococcus mutans* levels in oral cavity due to orthodontic treatment which causes ecological changes. The binding affinity of *Streptococcus mutans* to the orthodontic brackets is higher as compared to other bacterias.[2,3]

10%-35% of people around the world are undergoing Orthodontic treatment.[4] The presence of brackets, bands and other fixed appliances facilitate biofilm build up which leads to qualitative and quantitative changes in oral microbiota favouring enamel demineralisation and gingivitis.[5,6] According to various studies performed in the past, there is increase in the level of *Streptococcus mutans* among the patients with Orthodontic appliances that remains in the mouth for at least 1 or 2 years and results in irregular nature of the surfaces that promotes growth of cariogenic bacteria.[7]

A study by Peros K et al. [8] showed the significant increase in the level of *Streptococcus mutans* in the saliva after the orthodontic treatment was initiated, however first significant increase was detected after 6 weeks of treatment and highest levels were registered at 12th week of therapy.

A long-term usage of orthodontic appliances may promote the microbial flora with the increase risk of developing carious lesions. The potent pathogen involved in dental caries process includes mostly *Streptococcus mutans*.[9]

As there is increase in number of Orthodontic patients who are prone to oral diseases, various mouth rinses have been advocated as preventive measures during Orthodontic therapy. Each of these mouth rinses have their own mode of action and also the effectiveness.

II. MATERIALS AND METHODOLOGY:

It is an interdisciplinary study which was conducted to estimate the level of *Streptococcus mutans* before and during Orthodontic treatment. The study includes 30 male individuals (only male patients are selected as a standardisation method to avoid the influence of hormonal variation in the salivary composition) divided into six groups, each group consists of 5 male patients, 18-30 years of age group who are undergoing Orthodontic treatment and are willing to participate in the study.

Group 1: (Control group)- without use of any mouth rinse
Group 2: Chlorhexidine Gluconate mouth rinse (Hexidine)
Group 3: Amine Fluoride mouth rinse (Amflor)
Group 4: Essential oil-based mouth rinse (Listerine)
Group 5: Cetyl Pyridinium Chloride mouth rinse (Colgate Plax)
Group 6: Plain Salt Water

Written informed consent was obtained from patients who are enrolled for the study. Patients were instructed to brush their mouth twice a day using same brand toothpaste and tooth brush and use the appropriate mouth rinse twice a day. They were not allowed to eat or drink for 30 minutes after rinsing their mouth, once in the morning and once before going to sleep in the night. Patients with systemic conditions were not included in the study. Commercially available mouth rinses pertaining to the above-mentioned groups were used. Three Paraffin stimulated saliva samples were collected in sterile containers from each patient at different time intervals. First saliva sample was collected before starting the Orthodontic treatment (T0). Second sample after 45 days of starting the Orthodontic treatment (T1) and third sample was collected after 90 days of Orthodontic treatment (T2.).

All the saliva samples collected in sterile containers were labelled and packed with ice packs and transported within 2 hours of collection. Microbiological analysis of *Streptococcus mutans* was performed by culturing them on Mutans Sanguis agar (HiMedia). All the plates were incubated at 37°C for 24-48hours. Growth on agar plate were analysed by their colony morphology. *Streptococcus mutans* shows greyish yellow colour rough, heaped with irregular colonies resembling frosted glass which are approximately 0.5-2 mm in diameter. Number of colony forming units (CFU/mL) was noted down from respective samples, gram staining and biochemical analysis was done for all the isolates for the presumptive diagnosis of *Streptococcus mutans* by using tests to ferment mannitol, Sorbitol, arginine hydrolyses, ability to produce hydrogen peroxide and catalase reaction.

The results were analysed using IBM SPSS version 24. The statistical analysis of the study was done for three stages in all six groups. Mean, Median and Standard Deviation for each group was calculated. Friedman test and Kruskal Wallis test were used. The values are represented in number (n), Mean, median and SD (Standard deviation).

III. RESULTS

This study of 30 male patients, with an age group 18-30 years analysed the *Streptococcus mutans* level in oral cavity. Although, the *Streptococcus mutans* levels were reduced with the use of different mouth rinses there was no statistically significant difference found in the levels of *Streptococcus mutans*.(Figure-1)

The chi square values were obtained for each group, the p-value was $p>0.05$, which was not statistically significant. Mean and Median were compared for different groups, Group 1 showed slight decrease in *Streptococcus mutans* level at T1 and at the end of T2 it showed increase in the levels. In Group 2 initially there was decrease in the level of *Streptococcus mutans* at T1 and then there was slight increase in the levels of *Streptococcus mutans* at T2.(Table 1&2)

Group 3 showed slight increase in *Streptococcus mutans* level at T1, the levels of *Streptococcus mutans* were maintained at T2 as there was no increase in the levels. (Table 1&2)

In group 4 no changes were documented in the level of *Streptococcus mutans* at T0 and T1 but later at T2 there was increase in *Streptococcus mutans* level. (Table 1&2)

Group 4 and Group 5 showed increase in *Streptococcus mutans* level during the orthodontic treatment at different time intervals also.

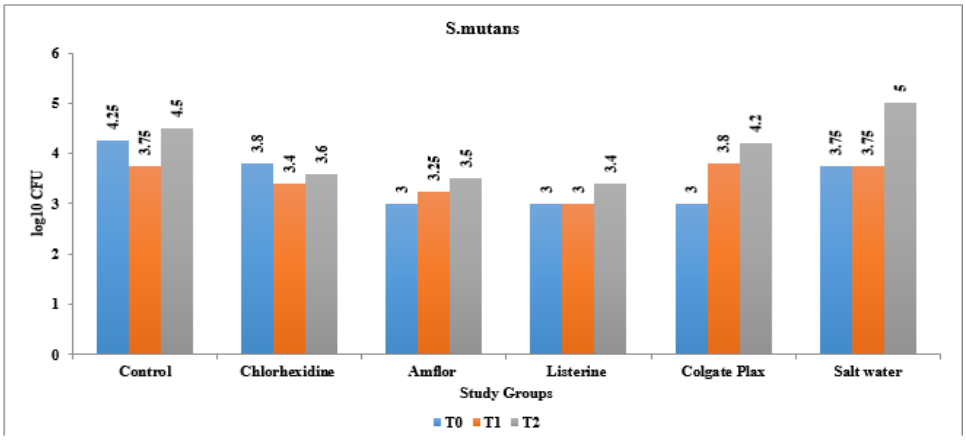

Figure-1: Comparison of effective mean levels of S.mutans in different groups at each time intervals.

Table 1:-comparison of log10 CFU S.mutans between different time intervals in each study group

Group	Time	N	Mean	SD	Min	Max	Percentiles			Friedman test	
							Q1	Median	Q3	Chi square value	p-value
Control	T0	4	4.25	1.50	2	5	2.75	5	5	4.67	0.10(NS)
	T1	4	3.75	1.26	2	5	2.5	4	4.75		
	T2	4	4.50	1.00	3	5	3.5	5	5		
Chlorhexidine	T0	5	3.80	0.84	3	5	3	4	4.5	1.08	0.58(NS)
	T1	5	3.40	1.14	2	5	2.5	3	4.5		
	T2	5	3.60	1.14	2	5	2.5	4	4.5		
Amflor	T0	4	3.00	0.82	2	4	2.25	3	3.75	0.57	0.75(NS)
	T1	4	3.25	0.96	2	4	2.25	3.5	4		
	T2	4	3.50	1.29	2	5	2.25	3.5	4.75		
Listerine	T0	5	3.00	1.00	2	4	2	3	4	0.40	0.82(NS)
	T1	5	3.00	1.41	2	5	2	2	4.5		
	T2	5	3.40	1.52	2	5	2	3	5		
Colgate Plax	T0	5	3.00	1.23	2	5	2	3	4	3.86	0.15(NS)
	T1	5	3.80	1.30	2	5	2.5	4	5		
	T2	5	4.20	0.84	3	5	3.5	4	5		
Salt water	T0	4	3.75	0.96	3	5	3	3.5	4.75	4.91	0.09(NS)
	T1	4	3.75	1.26	2	5	2.5	4	4.75		
	T2	4	5.00	0.00	5	5	5	5	5		

*p<0.05 statistically significant, p>0.05 Non Significant, NS

Table 2:-comparison of log10 CFU S.mutans between different study group in each time intervals

Time	Group	N	Mean	SD	Min	Max	Percentiles			Kruskal wallis test	
							Q1	Median	Q3	Chi square value	p-value
T0	Control	4	4.25	1.50	2	5	2.75	5	5	5.15	0.40(NS)
	Chlorhexidine	5	3.80	0.84	3	5	3	4	4.5		
	Amflor	4	3.00	0.82	2	4	2.25	3	3.75		
	Listerine	5	3.00	1.00	2	4	2	3	4		
	Colgate Plax	5	3.00	1.23	2	5	2	3	4		
	Salt water	4	3.75	0.96	3	5	3	3.5	4.75		
T1	Control	4	3.75	1.26	2	5	2.5	4	4.75	1.85	0.87(NS)
	Chlorhexidine	5	3.40	1.14	2	5	2.5	3	4.5		
	Amflor	4	3.25	0.96	2	4	2.25	3.5	4		
	Listerine	5	3.00	1.41	2	5	2	2	4.5		
	Colgate Plax	5	3.80	1.30	2	5	2.5	4	5		
	Salt water	4	3.75	1.26	2	5	2.5	4	4.75		
T2	Control	4	4.50	1.00	3	5	3.5	5	5	7.12	0.21(NS)
	Chlorhexidine	5	3.60	1.14	2	5	2.5	4	4.5		
	Amflor	4	3.50	1.29	2	5	2.25	3.5	4.75		
	Listerine	5	3.40	1.52	2	5	2	3	5		
	Colgate Plax	5	4.20	0.84	3	5	3.5	4	5		
	Salt water	4	5.00	0.00	5	5	5	5	5		

*p<0.05 statistically significant, p>0.05 Non Significant, NS

IV. DISCUSSION

In order to maintain better oral hygiene among patients during their fixed orthodontic treatment many orthodontists prescribe various adjuncts like mouth rinses, pastes etc.[10] In this study, the analysis of *Streptococcus mutans* level in oral cavity was assessed with prescription of different types of mouth rinses.

Presence of orthodontic appliances may produce a transitory increase of bacterial concentration and isolation rate of oral streptococci. According to various past studies performed during Orthodontic treatment, there is significant increase in salivary flow rate, saliva Ph and buffer capacity leading to loss of minerals and making the tooth surface irregular.[11] Formation of irregular surfaces it promotes the growth of acidogenic bacteria which prefers hard surface to grow.[12] Saliva pellicle plays an important role around the brackets during fixed orthodontic treatment which also increases *Streptococcus mutans* level in oral cavity.[13,14]

The result of this study shows, highest mean and median is 4.25 and 5 respectively of control group and lowest mean and median is 3.00 and 3 respectively of Amflor group and listerine group before the start of the orthodontic treatment. After the use of different mouth rinses and standard toothbrush and toothpaste there was decrease in mean from 3.80 at T0 to 3.40 at T1 and median from 4 at T0 to 3 at T1 in chlorhexidine group. Whereas listerine group shows no difference in mean of 3.00 from T0 to T1, but median is decreased from 3 at T0 to 2 at T1. Amflor group shows slight increase in mean value of 3.25 at T1 to 3.50 atT2, but median of 3.5 at T1 and T2 shows no difference. On the other hand, other groups show increase in mean and median from T1 to T2 with the highest mean of 5.00 and median of 5 at T2 in Plain Salt Water group and the lowest mean of 3.40 and median 0f 3 in listerine group.

Chlorhexidine, Amflor and Listerine mouth rinses are effective in reducing the levels of *Streptococcus mutans* during orthodontic treatment, mouth rinses used in proper way can be very effective in maintaining oral hygiene.

Jessica E. Koopman et al.[15] observed that amine fluoride(AmF) combined with stannous fluoride(SnF_2) mouthwash was used to reduce the caries and demineralization.[16] Van Loveren et al.[17] observed that there was no significant difference in bacterial composition with the use of AmF/SnF2 mouth rinse,this is similar to our findings.

Tufekci et al.[18] showed that routine use of Listerine mouth rinse can prevent accumulation of dental plaque and gingivitis that are common in orthodontic patients. Pahwa et al.[19]observed that 0.07% of cetylpyridium mouth rinse was effective in reducing bleeding and plaque index scores during orthodontic treatment. A study from Turkey by Emel Sari and Ilhan Birinci reported that, 0.2% chlorhexidine gluconate proved to be an important therapeutic agent in controlling *Streptococcus mutans* levels in patients with fixed orthodontics appliances.[20]

Another study by Alves PV et al,[21] reported that the level of *Streptococcus mutans* in saliva was high before using 1% chlorhexidine gel and 0.05% sodium fluoride solution as mouth rinses, but showed a significant difference after its usage as the enamel surface showed signs of remineralisation.

Nowadays chemical plaque control agents are being used to enhance the effect of plaque control in orthodontic therapy, Shreya Shruti Shah et al,[22] Observed that chlorhexidine had significantly decreased the plaque indices and can be used as chemical plaque controlling agent.

Various studies performed during Orthodontic treatment shows that mouth rinses containing chlorhexidine in combination with sodium fluoride has a significant improvement in oral hygiene status of orthodontic patients.[23]

A systemic review was conducted which proves that the use of oral mouth rinses in patients with fixed orthodontic appliances shows evidence of effectiveness in controlling the cariogenic plaque.[24,25]

In this study, these commercially available mouth rinses have shown clinical effectiveness and exhibited antimicrobial effect in reduction of bacterial concentration.

V. CONCLUSION

As a preventive method to maintain oral hygiene during orthodontic treatment the use of mouth rinses are essential which helps in decreasing the levels of *Streptococcus mutans*.

The listed mouth rinses had varied effectiveness at different time interval. However, the use of mouth rinses is highly recommended during Orthodontic treatment, as its frequent use can reduce the levels of *Streptococcus mutans*. Our study findings show that, use of mouth rinses has a clinically significant reduction in the occurrence of dental decay/white spot lesions during Orthodontic therapy.

It is recommended to use mouth rinses during orthodontic treatment because of their effectiveness and better results than any other adjunct in maintaining oral hygiene.

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