Original Article

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Effect of antimicrobial agents on the oral microflora in patients undergoing fixed orthodontic therapy–An *ex vivo* comparative analysis

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Abstract:

Aim: To evaluate and compare the efficacy of Ozonated Olive Oil Gel, Chlorhexidine gel, and Amflor (Fluoridated) mouthwash on reducing the count of Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy evaluated at different time intervals.

Methods: Sixty patients undergoing orthodontic treatment were randomly divided into three groups (n = 20) based on antimicrobial agents used (Group 1: Ozonated olive oil gel; Group 2: Chlorhexidine gel; Group 3: Fluoridated mouthwash). Elastomeric modules from brackets were collected at T_0 (Fresh samples) and T_1 (2nd week) and T_2 (4th week) for assessment of the microbial growth. These collected modules were cultured and evaluated for the presence of Streptococcus Mutans and Lactobacilli and numbers of colonies were counted at each interval. Data obtained was subjected to statistical analysis using SPSS software (Version 20.0). Level of significance was kept at 5%. Intra-group and inter-group comparison between pretreatment, 2nd week and 4th week was done for each group using Wilcoxon signed rank test and Mann–Whitney U test.

Results: There was presence of Streptococcus Mutans and Lactobacilli during orthodontic treatment which progressively increased from T_0 to T_1 and then declined from T_1 to T_2. The colony counts were maximum for Fluoridated mouthwash and least for Chlorhexidine and the results were statistically significant ($P < 0.05$).

Conclusion: All three antimicrobial agents used were effective against Streptococcus mutans and Lactobacillus. Chlorhexidine proved to be more efficacious whereas Fluoridated mouthwash proved to be least effective against both Streptococcus mutans and Lactobacillus bacteria.

Keywords:

Antimicrobial agents, chlorhexidine, lactobacillus, ozonated olive oil gel, streptococcus mutans

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Introduction

Today, with an ever-growing emphasis on aesthetics, orthodontic treatment is becoming increasingly popular among adults.[1] The fixed orthodontic appliances have long been associated with an increase in plaque accumulation, bacterial colonization, and resultant enamel decalcification. Orthodontic appliances can alter the coronal anatomy of the tooth, thereby leading to an increased number of retentive surfaces and posing a difficulty in controlling the formation and adhesion of plaque.[2]

The dental literature reports that orthodontic treatment induces changes in the oral environment by increasing the number of retentive surfaces for plaque, augmenting the bacterial levels of Streptococcus mutans and Lactobacillus. These appliances also

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modify the patient's salivary characteristics, that are, pH, buffer capacity, and salivary flow.[3] Enamel demineralization after placing orthodontic appliances can affect more than 50% of patients. The process is found most frequently in cervical and middle thirds of the buccal surfaces of upper lateral incisors, lower canines, and first premolars.[4]

These changes in the oral environment deteriorate further by the use of attachments such as elastic chains, loops, and springs. Elastomeric ligatures have been found to harbor many microorganisms.[5] Researchers have attempted to consistently evaluate the efficacy of these materials and several studies have been conducted on these ligatures to assess for microbial colonization.

With the advancements in material sciences, orthodontic bonding materials and appliances have evolved rapidly. Some of the major developments in the recent times have been the advent of self-ligation brackets, use of fluoride releasing adhesives and even modules.[6] Elastomeric ties have been thought to be the main culprit in harboring microorganisms and there is elimination of these ties in self-ligating brackets.

Over the years, various plaque control methods have been advocated. Some studies recommend scrupulous oral cleanliness, suggesting the use of irrigators, electrical or ultrasonic brushes, rinsing, varnish applications, use of antimicrobial agents, but one of the most important requirements for oral health is the motivation of the patient.[7]

Chlorhexidine in mouthwash form is used to reduce oral bacterial load. Chlorhexidine used in different forms has bacteriostatic effects and is effective in decreasing plaque by limiting adhesion between bacteria and enamel and in term affecting the formation of enamel film.[8]

Daily use of fluoridated mouthwashes containing sodium fluoride has also shown to result in a significant decrease in the development of carious lesion around and beneath bands. Benson carried out a systematic review and recommended the daily use of 0.05% NaF mouthwash to prevent enamel demineralization during fixed orthodontic treatment.[9]

One of the latest methods advocated for control of microflora in the oral environment is the use of ozonized olive oil gel. Ghobashy *et al*.[10] evaluated the effects of ozonized olive oil gel in reducing enamel demineralization around orthodontic bracket during orthodontic treatment. The use of ozonized olive oil gel in addition to the standard oral hygiene regimen was found to show significantly less decalcification of teeth among orthodontic patients. Although ozonized olive oil gel may be advocated for the control of microflora, there is little in orthodontic literature regarding its effect, specifically on Streptococcus mutans and Lactobacillus, which form the main component of dental plaque and are involved in periodontal diseases as well as dental decay.[11] Also, there is no study comparing the efficacy of Ozonized Olive Oil Gel vis-a-vis Chlorhexidine and Fluoridated mouthwash.

Hence the current study was designed and conducted to evaluate and compare the efficacy of Ozonated Olive Oil Gel, Chlorhexidine gel, and Amflor (Fluoridated) mouthwash on reducing the count of Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy evaluated at different time intervals. The null hypothesis was that there is no significant difference in the efficacies of ozonated olive oil gel, chlorhexidine gel, and Amflor mouth wash on reducing Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy as evaluated at different time interval.

Materials and Method

The present study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, I. T. S. Center for Dental Studies & Research, Muradnagar to evaluate and compare the efficacy of Ozonated Olive Oil Gel, Chlorhexidine gel, and Amflor (Fluoridated) mouthwash on reducing the count of Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy evaluated at different time intervals. The study was approved by the Institutional Ethical Review Board.

Sample

Sixty orthodontic patients undergoing fixed mechanotherapy who were willing to take part in the study were randomly selected.

Inclusion criteria

1. Patients undergoing Fixed orthodontic treatment with 0.022" MBT conventional metal brackets.
2. Age group of 15–30 years
3. Patients were brushing twice daily.

Exclusion criteria

1. Subjects who had taken a course of antibiotics in the previous 3 months.
2. History of smoking, periodontal disease, systemic disease, pregnancy and lactation, dental treatment (restorations, crown, and bridge)
3. History of use of mouthwash, hypersensitivity to mouthwash
4. History of parafunctional and deleterious habits (tobacco chewing)

Sample group

Twenty patients each were randomly allocated (Lot of Draws) into three groups based on the type of antimicrobial agent regimen to be used.
Group 1: Ozonated olive oil gel application
Group 2: Chlorhexidine gel (Hexigel)
Group 3: Fluoridated mouthwash (Amflor)

Method

Patients selected were undergoing fixed orthodontic therapy with stainless steel MBT (0.022″ × 0.028″) prescription (Victory Series, 3M-Unitek, United States). Oral prophylaxis was carried out in all the patients. Arch-wire was ligated with the elastomeric modules in the assigned groups.

The Protocol for the antimicrobial application was as follows:

1. Ozonated Olive Oil Gel: The selected teeth were dried and the gel was applied with the cotton pellet on the buccal surface. Patient were instructed not to eat or drink for 30 min and then instructed to rinse thoroughly. The procedure was repeated at initial time T_0 and at each subsequent week for 4 weeks
2. Hexigel: selected teeth were dried and gel was applied with the cotton pellet. After 10 min, patients were instructed to rinse thoroughly. The procedure was repeated at initial time T_0 and at each subsequent week for 4 weeks
3. Amflor mouthwash: Patients were instructed to take 20 ml of mouthwash into a cup and dilute the solution with equal amount of water and swish for 30 s and spit the solution out. Patients were also instructed to eat or drink only after rinsing with water after 20 min had elapsed. This was prescribed as a home procedure and written instructions were given for the same. Patients were asked to use mouth rinse twice daily.

All the patients were recalled after 2nd week and modules were aseptically and carefully removed from mandibular left premolar in 50% patients and mandibular right premolar in 50% patients and were placed in 0.5 ml of phosphate buffered saline in the Eppendorf tube [Figures 1a, b and 2]. New modules were placed after the removal from the tooth. Similarly, sample collection was done after 4th week. Samples of modules in the packed state from the manufacturer were considered as the pre-treatment sample for checking the presence or absence of Streptococcus mutans and Lactobacillus. All the samples were properly labelled according to the groups.

Sample collection was done at time intervals:

T0: Module samples in the packed state from the manufacturer (pre-treatment)

T1: Sample collection at the end of 2nd week taken from left Premolar in 50% patients and from the right Premolar in remaining 50% patients, respectively.

T2: Sample collection at the end of 4th-week taken from right premolar in 50% patients and from the left premolar in 50% of the patients, respectively (Contralateral premolar for Group 1).

Samples were tested for both the presence of Streptococcus mutans (Subgroup A) and Lactobacillus (Subgroup B), thus the final groups and subgroups for the microbial evaluation are depicted in Table 1.

For the microbial count, samples were vortexed for 10 s and a series of three 10-fold (10^{-3} dilution) of each sample were prepared. Ten milliliters of each dilution were seeded on to the Mitis Salivarius and Rogosa SL agar plates for Streptococcus mutans and Lactobacillus estimation, respectively. Plates for the Streptococcus mutans estimation were incubated in an incubator at 37°C with 5–10% CO2 for 24–48 h. For Lactobacillus estimation, the plates were incubated at 37°C for 48–72 h at 5% CO_2 in the vacuum anerobic jar. Plates were removed from the jar; growth was noted, and the number of colonies was counted with a colony counter.

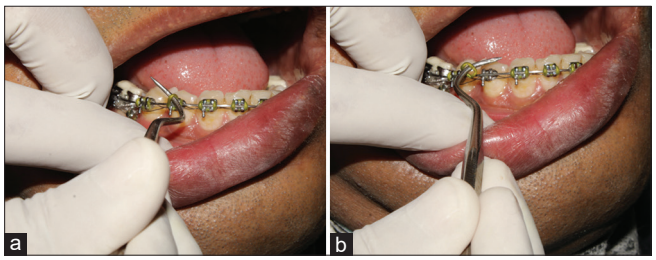

Figure 1: (a and b) Removal of elastomeric module from mandibular premolar bracket

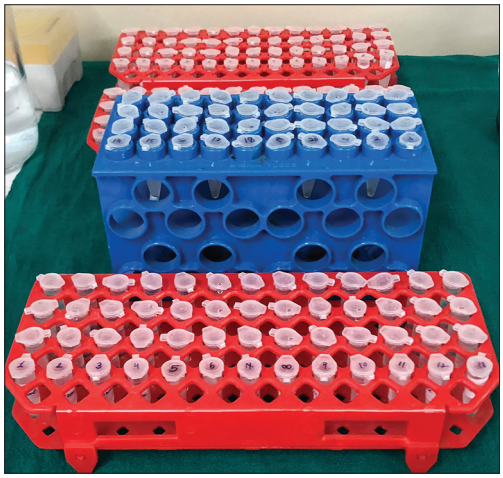

Figure 2: Sample collection

The data was expressed as number of colonies per sample and tabulated and sent for statistical analysis [Figures 3a–f and 4a–c].

Statistical analysis

Data obtained was subjected to statistical analysis using SPSS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). $P < 0.05$ was considered significant and $P < 0.01$ was considered to be highly significant.

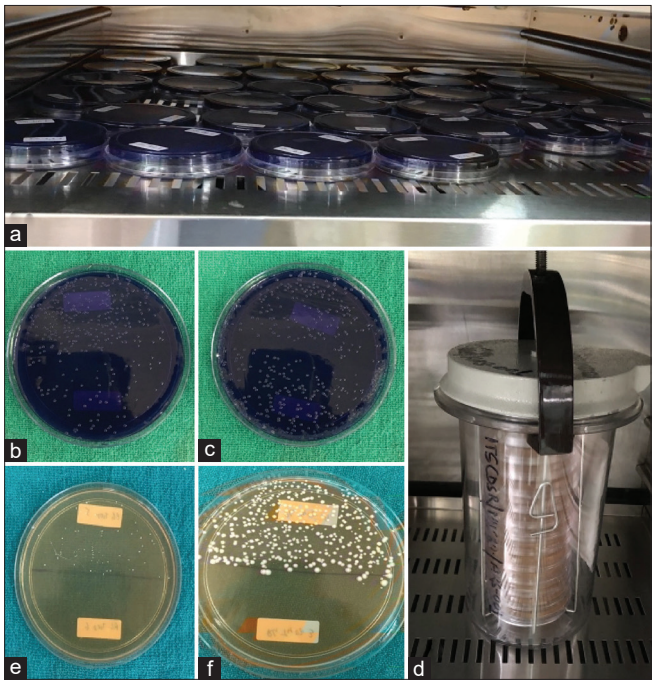

Figure 3: (a) Culture of Streptococcus mutans (b and c) Colony of Streptococcus mutans using Mitis Salivarius Agar (d) Anaerobic culture of Lactobacillus using anaerobic gas jar (e and f) Colony of Lactobacillus using Rogosa SL Agar

Level of significance was kept at 5%. Intra-group and intergroup comparison between pretreatment, 2nd week and 4th week was done for each group using Wilcoxon signed rank test and Mann–Whitney U test.

Results

Table 2 depicts the descriptive analysis of both the subgroup A and B for Group 1, 2, and 3 at T_0, T_1, and T_2. [Table 2]

Table 3 shows intra-group comparison of group 1, 2, and 3 using Wilcoxon Signed rank test. For subgroup A and B, bacterial count significantly increased ($P < 0.05$) from T_0 to T_1 in all the three groups and there was a significant ($P < 0.05$) decline seen in the bacterial count from T_1 to T2. [Table 3]

Intergroup comparison for each of the subgroups was done using Mann–Whitney U test. For Subgroup A, at T_0–T_1 microbial count showed significant increase ($P < 0.05$) in Group 3 and least in Group 1, whereas from T_1 to T_2 microbial count reduction was highly significant ($P < 0.01$) in Group 2. For Subgroup B, at

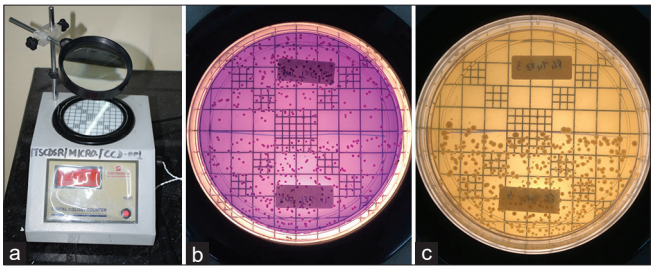

Figure 4: (a) Colony Counter (b) Counting the colony of Streptococcus mutans using colony counter (c) Counting the colony of Lactobacillus using colony counter

Table 1: Groups and Subgroups for the Microbial evaluation

Groups	T_0 (standard or all samples)	T_1 (2nd week)	T_2 (4th week)
Group 1 (Ozonated Olive Oil Gel)	Group 1 A (T_0)	Group 1 A (T_1)	Group 1 A (T_2)
	Group 1 B (T_0)	Group 1 B (T_1)	Group 1 B (T_2)
Group 2 (Hexigel)	Group 2 A (T_0)	Group 2 A (T_1)	Group 2 A (T_2)
	Group 2 B (T_0)	Group 2 B (T_1)	Group 2 B (T_2)
Group 3 (Amflor mouth wash)	Group 3 A (T_0)	Group 3 A (T_1)	Group 3 A (T_2)
	Group 3 B (T_0)	Group 3 B (T_1)	Group 3 B (T_2)

Table 2: Descriptive analysis for the Groups 1,2 and 3

Subgroup	Parameters	Group 1			Group 2			Group 3		
		T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
Subgroup A	Maximum	0	589	140	0	343	261	0	542	248
	Minimum	0	11	10	0	15	13	0	55	51
	Mean	0	134.8	82.7	0	175.85	64.65	0	195.85	131.75
	Standard Deviation	0	123.99	32.87	0	99.85	56.65	0	122.44	61.20
Subgroup B	Maximum	0	76	66	0	92	78	0	134	112
	Minimum	0	0	2	0	0	2	0	0	0
	Mean	0	30.9	23.95	0	34.25	30.1	0	28	27.9
	Standard Deviation	0	20.29	20.00	0	28.10	25.74	0	40.03	34.67

Table 3: Intra Group Comparison of Group 1,2 and 3 with Subgroup A and B using Wilcoxon Signed rank test

Groups	Subgroups	Time Interval	Mean	SD	SE	*Z* value	*P*
Group 1 (Ozonated Olive Oil Gel)	Streptococcus mutans	At pre-treatment (T_0)	0.00	0.00	0.00	3.920 (T_0-T_1)	<0.001*** (T_0 - T_1)
		2 weeks (T_1)	134.80	123.99	27.73	3.992 (T_0-T_2)	<0.001*** (T_0-T_2)
		4 weeks (T_2)	82.70	32.87	7.35	2.352 (T_1-T_2)	0.019 ** (T_1-T_2)
	Lactobacillus	At pre-treatment (T_0)	0.00	0.00	0.00	3.824 (T_0-T_1)	<0.001***(T_0-T_1)
		2 weeks (T_1)	30.90	20.29	4.54	3.922 (T_0-T_2),	<0.001*** (T_0-T_2)
		4 weeks (T_2)	23.95	20.00	4.47	1.868 (T_1-T_2)	0.062*(T_1-T_2)
Group 2 (Hexigel)	Streptococcus mutans	At pre-treatment (T_0)	0.00	0.00	0.00	3.920 (T_0-T_1)	<0.001***(T_0 -T_1)
		2 weeks (T_1)	175.85	99.85	22.33	3.920 (T_0-T_2),	<0.001*** (T_0-T_2)
		4 weeks (T_2)	64.65	56.65	12.67	3.342 (T_1-T_2)	0.001** T_1-T_2)
	Lactobacillus	At pre-treatment (T_0)	0.00	0.00	0.00	3.824 (T_0-T_1),	<0.001***(T_0-T_1).
		2 weeks (T_1)	34.25	28.10	6.28	3.921 (T_0-T_2),	<0.001*** (T_0-T_2)
		4 weeks (T_2)	30.10	25.74	5.76	2.316 (T_1-T_2)	0.021** (T_1- T_2)
Group 3 (Amflor Mouthwash)	Streptococcus mutans	At pre-treatment (T_0)	0.00	0.00	0.00	3.920 (T_0-T_1)	<0.001*** (T_0 -T_1)
		2 weeks (T_1)	195.85	122.44	27.38	3.921 (T_0-T_2)	<0.001*** (T_0-T_2)
		4 weeks (T_2)	131.75	61.20	13.69	2.837 (T_1-T_2)	0.005 ***(T_1- T_2)
	Lactobacillus	At pre-treatment (T_0)	0.00	0.00	0.00	3.824 (T_0-T_1)	<0.001*** (T_0 -T_1)
		2 weeks (T_1)	28.00	40.03	8.95	3.825 (T_0-T_2),	<0.001*** (T_0-T_2)
		4 weeks (T_2)	27.90	34.67	7.75	0.751 (T_1-T_2)	0.453* (b/w T_1-T_2)

*P>0.05 (Non-significant); **P<0.05 (Significant); ***P<0.01 (Highly significant)

T_0–T_1 increase in microbial count is significant ($P < 0.05$) in all the groups, whereas at T_1–T_2, group 1 and group 2 shows significant reduction ($P < 0.05$) and group 3 shows non-significant changes ($P > 0.05$). [Table 4].

Discussion

The current study was done to evaluate and compare the efficacy of Ozonated Olive Oil Gel, Chlorhexidine gel, and Amflor (Fluoridated) mouthwash on reducing the count of Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy evaluated at different time intervals. The null hypothesis set at the outset was that there is no significant difference in the efficacies of the three antimicrobial agents on reducing Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy as evaluated at different time interval. The results of the study were able to reject the null hypothesis.

Despite considerable advances in orthodontics, the formation of a favorable substratum for bacterial adhesion to orthodontic materials during orthodontic therapy remains an unresolved problem for the scientific fraternity. Bonded orthodontic brackets block exposure to good oral hygiene and create microbial nests, resulting in plaque accumulation.[12] The characteristic design and surface of both the orthodontic attachment and the composite can influence plaque retention. In addition to providing new retention areas for bacterial colonization, patients with orthodontic devices may also undergo oral ecological changes such as low salivary pH and increased retention of food particles, which in turn may contribute to increased rates of salivary Streptococcus mutans.[5]

The appliance architecture, specifically, the arch-wire ligation method is an additional factor in influencing bacterial colonization. Arch-wire ligation can be done using stainless steel ligature wires or elastomeric modules. Orthodontists prefer elastomeric ligatures as they are time saving, patient friendly, easy for application; have an aesthetic appearance and also have a potential for fluoride release. Some studies have shown that the elastomeric ligature exhibit bacterial plaque on its surface, with a higher number of microorganisms than can be verified on tooth surfaces because of its rough surface and the absorption properties of this material. It has also been reported that teeth attached with elastomeric ligature have slightly more microorganisms (S. mutans and Lactobacilli) than teeth attached with steel ligature wires, but the differences were not statistically significant and could be ignored.[13]

Studies have shown that patients undergoing orthodontic treatment show a continuing increase of Streptococcus mutans and Lactobacillus levels at diverse degrees of significance. According to Rosenbloom,[14] the highest levels of bacteria appear during the "active treatment" stage. Studies have also shown that in spite of the introductory period of oral hygiene instruction and training, combined with dietary advice, the levels of S. mutans in saliva increased significantly during the first 6 months of active orthodontic treatment. This observation is also supported by Corbett *et al.* (1981),[11] who showed a significant increase of S. mutans in microbial plaque from orthodontic patients.

It has been reported that Lactobacilli and Streptococci species create a low pH oral environment (pH < 5.5)

Table 4: Inter Group Comparisons For each Subgroups using Mann-Whitney U test

Comparison	Subgroups	Time Interval	Group	Mean	SD	SE	*Z* value	*P*
Group 1 (Ozonated olive oil gel) Vs. Group 2 (Hexigel)	Streptococcus mutans	2 weeks	Ozone	134.80	123.99	27.73	1.610	0.107*
			Hexigel	175.85	99.85	22.33		
		4 weeks	Ozone	82.70	32.87	7.35	2.192	0.028**
			Hexigel	64.65	56.65	12.67		
	Lactobacillus	2 weeks	Ozone	30.90	20.29	4.54	0.080	0.935*
			Hexigel	34.25	28.10	6.28		
		4 weeks	Ozone	23.95	20.00	4.47	0.514	0.607*
			Hexigel	30.10	25.74	5.76		
Group 1 (Ozonated Olive oil. gel) Vs. Group 3 (Amflor Mouthwash)	Streptococcus mutans	2 weeks	Ozone	134.80	123.99	27.73	1.880	0.060*
			Amflor	195.85	122.44	27.38		
		4 weeks	Ozone	82.70	32.87	7.35	2.342	0.019**
			Amflor	131.75	61.20	13.69		
	Lactobacillus	2 weeks	Ozone	30.90	20.29	4.54	1.949	0.051*
			Amflor	28.00	40.03	8.95		
		4 weeks	Ozone	23.95	20.00	4.47	0.447	0.655*
			Amflor	27.90	34.67	7.75		
Group 2 (Hexigel) vs. Group 3 (Amflor Mouthwash)	Streptococcus mutans	2 weeks	Hexigel	175.85	99.85	22.33	0.162	0.871*
			Amflor	195.85	122.44	27.38		
		4 weeks	Hexigel	64.65	56.65	12.67	3.585	<0.001***
			Amflor	131.75	61.20	13.69		
	Lactobacillus	2 weeks	Hexigel	34.25	28.10	6.28	1.489	0.137*
			Amflor	28.00	40.03	8.95		
		4 weeks	Hexigel	30.10	25.74	5.76	0.745	0.457*
			Amflor	27.90	34.67	7.75		

*P>0.05 (Non-significant); **P<0.05 (Significant); ***P<0.01 (Highly significant)

because of the bacterial byproducts. S. mutans and Lactobacilli both are strongly associated with the initiation of dental caries, and have long been associated with its development.[4] Therefore, determination of both the S. mutans and the Lactobacilli counts was considered important to determine oral hygiene risk in our study. Also, whether the counts increase over a period of time was also evaluated. Although many preventive measures are available for the control of plaque and microbes; the most commonly used antiplaque agents are Chlorhexidine and Fluoride products such as gels or mouthwashes. Also there are some emerging products gaining popularity such as use of ozone in the form of gas, water, or gels.[15]

Chlorhexidine gluconate is a cationic biguanide with broad-spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental biofilm (plaque) and gingivitis have been demonstrated in several clinical studies. It is considered as the positive control (gold standard), to which all other anti-plaque agents should be compared. Its antibacterial action is because of an increase in cellular membrane permeability followed by coagulation of the cytoplasmic macromolecules. Some *in vitro* studies have shown that CHX gluconate has the best antimicrobial activity against streptococcal mutans, Lactobacilli, *E. coli*, and C. albicans.[8]

Fluoride acts primarily by decreasing the pH at which enamel demineralizes. Tooth enamel is composed of crystals of hydroxyapatite, a mineral form of calcium apatite ($Ca_{10}(PO_4).6(OH)_2$). Free fluoride ions can adsorb to hydroxyapatite crystals, inhibiting demineralization during acid challenge and enhancing remineralization when pH levels subsequently rise. Fluoride may also reduce acid production by inhibiting bacterial glucose metabolism and thus reducing acidogenesis and the associated enrichment of aciduric species in plaque. Usually, mouthwash must be used twice a week for about 1 min. But it is recommended that after mouthwash the patient must not eat, drink, and rinse, so the components of mouthwash are present for a long time.[16]

Ozone is another form of antimicrobial agent, and has gained popularity. Ozone gas around the orthodontic brackets and their application was defined as a new technique to minimize demineralization of the enamel. It was found that ozone could be held for a long time by making ozone dissolve in a virgin olive oil, under controlled reaction of cooling and pressure and obtained in gel form.[17] Studies have shown that ozonized olive oil gel has a significant potential to maintain the enamel content of Ca and *P* through its protective effect against decalcification of teeth although the Ca/P ratios (2.33) have not achieved sound enamel values. Since the Ca and *P* are the main components of hydroxyapatite crystals, ozonized olive oil gel can decrease the potential for decalcification during orthodontic treatment.[11]

Ozone can significantly reduce the number of Streptococcus mutans in plaque and effectively penetrate into the lesion and kill the great majority of microorganism, resulting in a delayed recolonization compared with enamel surface. Ozone was found to have a potent antibacterial effect explained by the fact that it causes disruption of the envelope integrity through peroxidation of phospholipids. The re-application of ozone could slow the recolonization pattern and achieve long-term suppression. Ozone enables the shifting of flora from acidogenic and acidouric microorganism to normal oral commensals, which will allow re-mineralization to occur.[10]

Many authors have stated the effect and efficacy of the antimicrobials available and their effect during orthodontic treatment but till now none of the studies have compared the efficacy of the conventional and gold standard antimicrobials like fluoride and Chlorhexidine with the ozonated olive oil gel in reducing the most commonly found microorganisms (Streptococcus mutans and Lactobacillus) around the retentive surfaces while orthodontic treatment at different intervals.

Hence this study was planned to evaluate and compare the efficacy of Ozonated Olive Oil Gel, Chlorhexidine gel, and Amflor mouthwash on reducing Streptococcus mutans and Lactobacillus in patients undergoing fixed orthodontic therapy as evaluated at different time intervals.

In this study, bacterial sampling was done at three different intervals T_0 (At pretreatment), T_1 (2 weeks), T_2 (4 weeks) of the elastomeric modules for the three groups of antimicrobial agents (Group 1- Ozonated Olive Oil Gel, Group 2- Hexigel, Group 3-Amflor mouthwash) and the samples were evaluated for the colonization of the Streptococcus mutans and Lactobacillus.

On comparison of all 3 groups, we found that there was a significant amount of bacterial growth for all the three groups but this value decreased from T_1 to T_2, that is, from 2 weeks till 4 weeks. This indicates that all 3 antimicrobial agents used were effective for both Streptococcus mutans and Lactobacillus. This result is in accordance with the study conducted by Rosembloom where he stated that patients undergoing orthodontic treatment show a continuing increase of Streptococcus mutans and Lactobacillus levels at diverse degrees of significance.[14]

For Streptococcus mutans subgroup, there was significant increase in the first 2 weeks although it was highest in the Amflor mouthwash regimen group and least in the Ozonated Olive Oil Gel group. At the end of 4 weeks, the bacterial count although significantly decreased in all the three groups but it was highly significant in the Hexigel group, whereas it was least in the Amflor Mouthwash group.

For Lactobacillus subgroup, from the time of archwire placement and at the 2nd week interval there was an increase in the Lactobacillus count in all the three antimicrobial group, whereas at the end of 4th week, the decrease in the count was significant in Hexigel group and Ozonated Olive Oil Gel group whereas Amflor mouthwash showed non-significant effect at between T_1 and T_2. Indurkar *et al.* stated that ozonated olive oil gel can be a comparable alternative of chlorhexidine against plaque which is in accordance with our study stating the comparable efficacies of Ozonated Olive Oil Gel and Chlorhexidine.[18]

This study estimated the bacterial adhesion only on the particular ligatures. Estimation of altered microflora in and around the environment of the ligature is a limitation of this study and could be pursued utilizing improved technologies, DNA isolation, and polymerase chain reactions.

Since Amflor mouthwash was used twice a week for 1 min in this study as suggested by Danaei *et al*., the efficacy of Amflor mouthwash needs to be further evaluated since some authors also suggest use of mouthwash daily.[19] Also various authors have reported that Chlorhexidine may cause brown staining of surface of teeth; this being one of its major drawbacks.[20]

Conclusion

Through the results it can be concluded that all 3 antimicrobial agents (Ozonated Olive Oil Gel, Chlorhexidine, and Amflor mouth wash) used were effective against Streptococcus mutans and Lactobacillus. Chlorhexidine proved to be more efficacious as compared to Ozonated olive oil gel. Amflor mouthwash showed the least effective results against both Streptococcus mutans and Lactobacillus bacteria.

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Conflicts of interest
There are no conflicts of interest.

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