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Comparative Evaluation of Anti-Microbial Efficacy of Alum Mouthwash and 0.2% Chlorhexidine Mouthwash in Reducing Streptococcus Mutans Biofilm from Conventional Metal Brackets - an in Vitro Study.

Running Title: Anti-Microbial Efficacy of Alum Mouthwash on Orthodontic Conventional Metal Brackets.

Dr. Ruchita D. Tayade[1] , Dr. Amit Nehete[2] , Dr. Nitin Gulve[3] , Dr. Mukul Tambe[4] , Dr. Vedant Gambhire[1] , Dr. Prerna Badgujar[1]

[1.] Student, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik.

[2.] Professor, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik

[3]. Head of the Department, Professor, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik.

[4.] Lecturer, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik.

Corresponding Author: Dr. Ruchita D. Tayade, Student, Department of Orthodontics and Dentofacial Orthopedics, M.G.V.'s K.B.H Dental College and Hospital, Nashik.

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KEYWORDS

chlorhexidine mouthwash,

alum mouthwash, biofilm,

streptococcus mutans,

orthodontic brackets, conventional metal brackets,

fixed orthodontic therapy.

ABSTRACT:

Introduction: A lot of individuals seek fixed orthodontic treatment to correct their misaligned teeth, where brackets plays a crucial role for passive components in the orthodontic process. Utilizing fixed orthodontic devices makes maintaining strict oral hygiene an important challenge. Chlorhexidine, the gold standard, used for cleaning fixed orthodontic appliances in reducing the plaque build-up on brackets, but cannot be advocated for routine use in orthodontic patients because of staining on teeth if administered for long-term, but might rather be used temporarily for patients with present inflammation or increased risk of gingival inflammation due to microbial burden. Potash Alum also known as 'Phitkary' has proved to be significant as Alum mouthwash in continued daily usage for decreasing the level of streptococcus mutants in saliva. The efficacy of a daily use of alum rinse has lead in reducing the pathogenicity of bacteria and also decreased bleeding and inflammation.

Objectives: To evaluate and compare anti-microbial efficacy of Alum and 0.2% Chlorhexidine mouthwashes in reducing Streptococcus mutants biofilm on conventional metal brackets.

Methods: A total of Forty-five conventional metal brackets 3M™ were randomly selected and sterilized by immersion in 1% NaOCl (sodium hypochlorite) for 30 minutes. After sterilization the brackets were removed with sterile forceps and rinsed thrice in sterile distilled water. The brackets were then placed in the plaque solution consisting of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion (BHI) broth and 5 ml of Streptococcus mutans and were incubated aerobically at 37^0 C for 24 hours. After cultivation of Streptococcus mutans biofilm, the brackets were removed and Colony forming unit baseline was calculated followed by dividing the brackets into 3 groups (15 brackets in each group were divided randomly) from which first group is the control group in which the brackets

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were soaked in artificial saliva for 1 minute and the rest two groups were subjected to the effects of Alum and 0.2% chlorhexidine mouthwash respectively for 1 minute. After 1 minute, the data was calculated for Steptococcus mutans biofilm. Quantitative assessment was carried out by comparing the number of viable colonies of Streptococcus mutans. One way Anova F test was used to compare the data between the experimental and control groups. ($p < 0.001$).

Results: There is a significant difference in colony forming unit count soaking conventional metal brackets with alum mouthwash and chlorhexidine mouthwash. Even though a highly significant result was seen with alum mouthwash, but when compared with gold standard chlorhexidine the results were still less significant than chlorhexidine mouthwash.

Conclusions: Streptococcus mutans colonies on conventional metal brackets can be reduced effectively by Chlorhexidine digluconate and Alum mouthwashes. 0.2% Chlorhexidine digluconate is more effective than Alum mouthwash.

1. Introduction

Many individual pursue fixed orthodontic therapy for fixing their mal-aligned teeth in which brackets plays an essential part of passive components used in orthodontic treatment. The use of fixed orthodontic appliances makes practicing strict oral hygiene a significant task[1].

Dental plaque is a complex biofilm, which begins with the formation of the salivary pellicle which develops gradually into a mature plaque. During orthodontic treatment, because of interaction with arch-wires and ligatures, plaque accumulates more in areas around brackets therefore, causing self-cleaning mechanism impossible in oral-cavity, promoting retention of dental biofilm. As a consequence, the patient is affected with changes in oral pH and development of caries. In the cariogenic process, the most common biofilm-associated oral infection worldwide, Streptococcus Mutans is a key player.[2]

Chlorhexidine Digluconate a 'Gold Standard', well-known for chemical plaque control since 1970s, suitable for cleaning fixed orthodontic appliances in reducing the plaque build-up on brackets.[3] Chemically, Chlorhexidine is a cationic compound, causing disruption of its external cellular components of the biofilm along with a rupture of bacterial cytoplasmic membranes. It is important to stress out that Chlorhexidine cannot be advocated for routine use in orthodontic patients because of brown staining on teeth, but might rather be used temporarily for patients with present inflammation or increased risk of gingival inflammation due to microbial burden.[4]

Potash Alum ($KAl(SO4)_2 \cdot 12H_2O$) is a naturally occurring compound with anti-microbial and antifungal properties.[5] In the subcontinent, it is referred to as 'Phitkary'. It is a common household item that is inexpensive, odorless, and safe in little amounts. The Food and Drug Administration (FDA) in the United States has authorized it as a food additive because of its low toxicity. Alum salts have exhibited anti-caries activity in a number of laboratory and animal experiments and was proved to be significant as alum mouthwash in continued daily usage for decreasing the level of streptococcus mutants in saliva.[6]

The efficacy of a daily use of alum rinse lead to reducing the pathogenicity of bacteria and decrease bleeding and inflammation.[7]

However, there is no published data on effectiveness of Alum mouthwash which has antibacterial properties to reduce Streptococcus mutans from conventional metal brackets.

Therefore, the present study aims to evaluate and compare the anti-microbial efficacy of 0.02M Alum mouthwash with 0.2% Chlorhexidine mouthwash to reduce Streptococcus mutans biofilm from conventional metal brackets.

2. Objectives

I) To evaluate the colony forming unit (CFU) count of Streptococcus mutans on conventional metal brackets before soaking it in mouthwash.

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II) To evaluate the colony forming unit (CFU) count of Streptococcus mutans on conventional metal brackets without cleaning, soaking it with artificial saliva.

III) To evaluate the colony forming unit (CFU) count of Streptococcus mutans on conventional metal brackets after soaking it into the Alum mouthwash.

IV) To evaluate the colony forming unit (CFU) count of Streptococcus mutans on conventional metal brackets after soaking it into the 0.2% Chlorhexidine mouthwash.

V) To compare the colony forming unit (CFU) count of Streptococcus mutans on conventional metal brackets after soaking in into the Alum mouthwash and 0.2% Chlorhexidine mouthwash.

3. Methods

This is an in-vitro study and was carried out in the Department of Orthodontics and Dentofacial Orthopaedics & Department of Microbiology and Pathology. The objectives of the study was to evaluate and compare the colony forming unit (CFU) of Streptococcus mutans on conventional metal brackets soaking it with artificial saliva, alum and chlorhexidine mouthwash respectively.

The sample size comprised of 45 randomly selected conventional metal brackets 3M™. Brackets were sterilized by immersion in 1% NaOCl (sodium hypochlorite) for 30 minutes. After sterilization the brackets were removed with sterile forceps and cleaned by three rinses in sterile distilled water. The brackets were then placed in the plaque solution which consisted of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion (BHI) broth and 5 ml of Streptococcus mutans and were incubated aerobically at 37^0 C for 24 hours.

After cultivation of Streptococcus mutans biofilm, the brackets were removed and Colony forming unit baseline was calculated. Later the contaminated metal brackets were randomly divided into 3 groups with each comprising of 15 samples and were immersed in the treatment solutions for 1minute as follows:-

GROUP A: Brackets without cleaning soaked in Artificial saliva. (Control Group)

GROUP B: Soaked metal brackets into the 0.02M Alum mouthwash.

GROUP C: Soaked metal brackets into the 0.2% Chlorhexidine mouthwash.

Brackets in Group 1 & 2 were soaked into 0.02M Alum and 0.2% Chlorhexidine mouthwash respectively for 1 minute and diluting immediately in a 5% phosphate buffer solution (PBS) for analysis after. The data was collected after the effects of both the mouthwashes.

Statistical Analysis

The Statistical analysis of ordinal data was obtained and performed using Statistical Package for Social

Science (SPSS) version 21 for Windows (SPSS Inc, Chicago, IL). Continuous quantitative data was expressed in mean and standard deviation respectively.

4. Results

The following results were seen:

Descriptive statistics: Table 1 depicts the Colony forming unit count of all 45 samples after soaking in artificial saliva, alum and chlorhexidine mouthwash for 1 minute.

Table 2 ; depicts the distribution of the effect of soaking in artificial saliva and different types of Mouthwashes i.e. Alum and Chlorhexidine mouthwash respectively in reducing streptococcus mutans biofilm on conventional metal brackets.

As shown in Table:- 2, for Group A, the mean Colony forming unit count was 11.06 with standard deviation of 3.97. The Colony forming unit count of Group B was found to be 4.80 with an standard deviation of 0.86. Group C had a Colony forming unit count of 1.66 with an SD of 1.18.

This clearly shows that Group A has the highest Colony forming unit count, while Group C has the least.

Table 1:- Master table- Colony forming unit count of all 45 samples after soaking in treatment solutions.

Sr No.	Group1 (Control)	Group 2 (Alum Mouthwash)	Group3 (Chlorhexidine Mouthwash)
1	07×10^5	04×10^5	01×10^5
2	09×10^5	05×10^5	02×10^5
3	11×10^5	06×10^5	01×10^5
4	11×10^5	04×10^5	04×10^5
5	09×10^5	05×10^5	03×10^5

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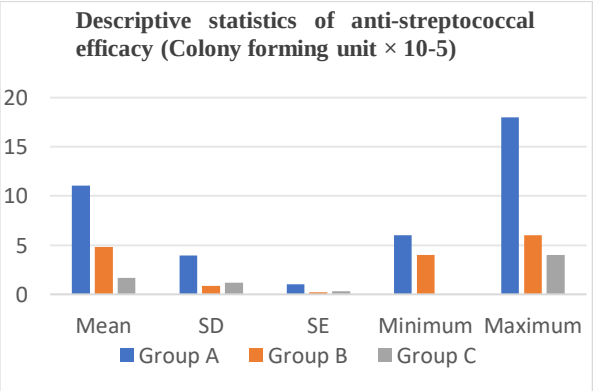
6	07 X 10^5	06 X 10^5	02 X 10^5
7	06 X 10^5	06 X 10^5	01 X 10^5
8	06 X 10^5	04 X 10^5	---
9	11 X 10^5	05 X 10^5	02 X 10^5
10	13 X 10^5	04 X 10^5	03 X 10^5
11	15 X 10^5	04 X 10^5	01 X 10^5
12	17 X 10^5	05 X 10^5	01 X 10^5
13	18 X 10^5	04 X 10^5	01 X 10^5
14	16 X 10^5	04 X 10^5	03 X 10^5
15	10 X 10^5	06 X 10^5	---

Table 2:Descriptive statistics of anti-streptococcal efficacy among the 3 groups.

	Mean	SD	SE	Minimum	Maximum
Group A No cleaning and with artificial saliva	11.06	3.97	1.03	6	18
Group B Alum mouthwash	4.80	0.86	0.22	4	6
Group C 0.2% Chlor-hexidine mouthwash	1.66	1.18	0.30	0	4

Graph 1: Shows the Mean and Standard deviation of group A, B, C respectively.



Inferential statistics:-

As shown in Table no.3, on Overall Comparison of the Colony forming unit count of Streptococcus mutans biofilm treated with different types of mouthwashes using One way Anova F test , a highly statistically significant ($p<0.001$) difference was observed among the three groups.

As shown in Table no.4, the pairwise comparative statistics among three study groups using Tukey's Post Hoc test showed that the differences in Colony forming unit count of the Alum group with control group was statistically highly significant ($p<0.001$).

On comparing the effect of the two mouthwashes i.e. alum and chlorhexidine mouthwash on the Streptococcus mutants biofilm on metal brackets, the result was found to be statistically significant ($p<0.05$).

Table 3:- Overall comparison of anti-streptococcal efficacy using One way Anova F test.

	Mean	SD	One way Anova F test	P value
Group A - No cleaning	11.06	3.97	57.575	p < 0.001**
Group B- Alum mouthwash	4.8	0.86		
Group C- 0.2% Chlorhexidine mouthwash	1.66	1.18		

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Graph 2: Shows that the least Colony forming unit count in Chlorhexidine than Alum mouthwash.

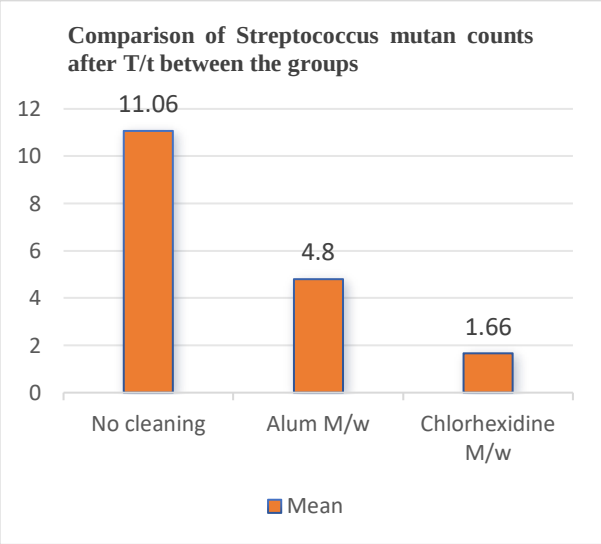
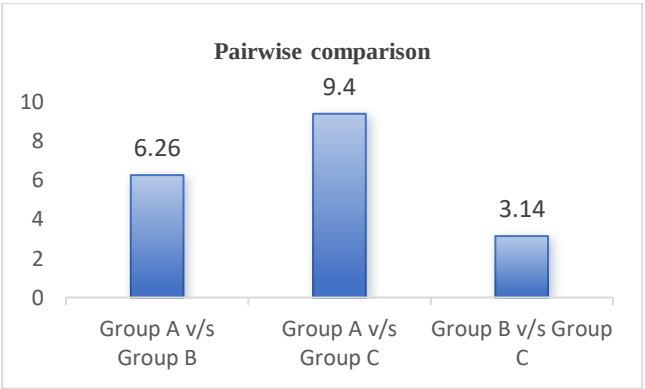


Table No:-4 Pair wise comparative statistics of anti-streptococcal efficacy among two different cleansing methods using Tukey's post hoc test.

Group	Comparison groups	Mean difference	P value, Significance
Group A No cleaning	**Group B** Alum mouthwash	6.26	p<0.001**
	Group C 0.2% Chlorhexidine mouthwash	9.4	p<0.001**
	Group C	3.14	p<0.05*
Group B Alum mouthwash	0.2% Chlorhexidine mouthwash		

p>0.05 – no significant difference *p<0.05 – significant

Graph 3: Shows a highly significant result for Alum, but compared with Chlorhexidine the result is significant.



5. Discussion

Careful oral hygiene practices are significantly hampered by fixed orthodontic appliances, which allow plaque to build up and remain stagnant around brackets and wires. In an attempt to seek a novel mouthwash that equivalent to Chlorhexidine, the current study aimed to evaluated and compare the antibacterial efficiency of Alum and Chlorhexidine mouthwashes.[1]

In the current study, a total sample size of 45 conventional metal brackets were placed in the plaque solution consisting of 15 ml of sterile sucrose, 30 ml of Brain Heart Infusion (BHI) broth and 5 ml of Streptococcus mutans and were incubated aerobically at 37^0 C for 24 hours. The brackets were then randonmly divided into 3 groups with 15 samples in each group in which the group A ,B C soaked in artificial saliva, alum and chlorhexidine mouthwash respectively for 1minute. Thereafter, the colony forming unit count was calculated for all the 3 groups. One way Anova F test was used to compare the data between the experimental and control groups. ($p < 0.001$).

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As shown in Table 2, the colony forming unit count was highest with Group A (control group) (11.06), which was the group soaked in artificial saliva, followed by Group B (Alum mouthwash) (4.80) and lowest with Group C (Chlorhexidine mouthwash) (1.66). On pairwise comparison among group A and group B the mean difference was 6.26 whereas among group A and group C was 9.4 ($p<0.001$**) which shows that the results are statistically highly significant. On the other hand, comparision between two mouthwashes (i.e. Group B and Group C), the mean difference was 3.14 ($p<0.05$) suggesting statistically significant difference between group B & C. The results concludes that conventional metal brackets soaked in Chlorhexidine mouthwash and alum mouthwash can reduce S.mutans biofilm with chlorhexidine digluconate more effective than Alum mouthwash. The reason for the current findings can be attributed to the mode of action of chlorhexidine mouthwash, alum mouthwash. One of the important property of chlorhexidine mouthwash is 'Substantivity' the ability to bind to oral tissue providing a prolonged bacteriostatic effect. On the other hand, alum mouthwash involves astringent and antimicrobial properties. The astringent effect occurs through the coagulation of the upper layers of tissue, leading to the development of a crust and the precipitation of cell membrane proteins on microorganisms.

Various studies have examined the effect of different mouthwashes on streptococcus mutants biofilm on metal, self-ligating as well as ceramic brackets. Singh AK et al in their study compared the antimicrobial efficacy of Chlorhexidine and Chlorine dioxide mouthwashes on Streptococcus mutans biofilm created on metal & ceramic self-ligating brackets concluded that Streptococcus mutans colonies on metal and ceramic self-ligating brackets can be reduced effectively by Chlorhexidine digluconate and Chlorine dioxide mouthwashes with chlorhexidine digluconate more effective for metal bracket group.[1]

Among orthodontic patients, there are reports that Chlorhexidine has been proven effective in reducing pathogenic bacteria associated with dental caries or reducing plaque/gingivitis. However, chlorhexidine mouthwash cannot be advised for long term in orthodontic patients because of brown staining on teeth which highlights the limitation of chlorhexidine mouthwash in orthodontic treatment.

Rinsing with a mixture of alum salt for oral health was advocated by Hippocrates in ancient times. Saltwater rinses are a very ancient, yet effective way of killing the bacteria in the mouth. It is well known that mouthwashes with a high salty concentration can kill bacteria by creating a hypertonic environment. Aluminum salts has demonstrated activity against oral bacteria. It has been widely used for its astringent properties**.** The mechanism of action is performed by the induction of coagulation in superficial tissue layer until the formation of crust followed by precipitation of the protein of cell membrane of the microbes.[8]

Vanishree BK et al in their study showed that components in the alum oral rinse were effective in decreasing plaque status. Hence, it could act as a substitute antimicrobial mouthwash.[5] Rupesh S et al found that children who used saturated saline rinses and alum rinses exhibited a significant decrease in S. mutans count after both 10 and 21 days. At the 21-day mark, the groups using saturated saline rinse and alum rinse demonstrated significant differences compared to the placebo rinse group. Additionally, the alum rinse group revealed a notable difference when compared to the saturated saline rinse group.[9]

Bihani SN et al conducted a study involving 40 children from municipal schools aged between 12 and 14 years to assess how effective daily supervised rinsing with a mouthwash formulated with alum is in reducing plaque and gingivitis. The results of this 2 week study demonstrate that a mouthwash containing 0.02 M Alum has significant effect on plaque inhibition. There was a reduction in gingivitis but it was not statistically significant.[10]

It should be stressed that as this was an in-vitro study, the test circumstances were not exposed to the changing electrolytes, microbes, or other highly changeable oral cavity elements that characterise the oral environment. Further in-vivo investigations are required to support these findings and randomized controlled trials can be done to conclude the strong evidence in support to this current findings.

6. Conclusion

It can be concluded that streptococcus mutans colonies on conventional metal brackets can be reduced effectively by Chlorhexidine digluconate and Alum

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mouthwashes. Even though a highly significant result was seen with alum mouthwash, but when compared with Gold Standard 'Chlorhexidine' (CHX), the results of alum mouthwash were still less significant than chlorhexidine mouthwash. 0.2% Chlorhexidine digluconate is more effective than 0.02M Alum mouthwash.

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