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A comparative evaluation of the antibacterial effects of commonly prescribed mouth rinses on streptococcus mutans in orthodontic patients

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Abstract

Aims: it is to camp are and evaluate the antibacterial effects of commonly prescribed mouth rinses on Streptococcus Mutans in orthodontic patients.

Materials and Methods: Thirty patients (12-20 years old) on fixed orthodontic treatment, with desirable oral health were subjected to Distilled water (Control), 0.2 % Chlorhexidine Gluconate (Clohex), Herbal (Hiora) and 0.1% Chlorine Dioxide (Freshclor) mouth rinse use respectively. Subsequently samples (elastic rings around the brackets) were collected from each patient at four different time intervals according to mouthwash used so as to create four mouthwash groups of 30 sample each. Samples were cultured and streptococcus mutans colonies were counted.

Statistical Analysis: Kruskal-Wallis Test and Mann Whitney 'U' Test was used and "p" value was calculated.

Results: Comparison of Mean CFU/ml (S. mutans) among Clohex, Freshclor, Hiora mouth rinses and control (distilled water rinses) groups was done. It was found minimum in Clohex mouth rinse 131.57±30.42 and highest in Freshclor mouth rinse 173.43±42.19. It was 150.57±43.84 in Hiora mouth rinse, while control group had highest count 232.30±31.21. Statistically highly significant difference was found between all three mouth rinses and control groups in respect to Mean CFU/ml ($P<0.001$).

Conclusion: Chlorhexidine was found most effective against S. mutans followed by Hiora, Freshclor, and distilled water mouthwash respectively.

Keywords: Mouthwash, 0.2 % Chlorhexidine Gluconate, 0.1% Chlorine Dioxide, Herbal mouthwash, Streptococcus mutans.

Introduction

Now days there is a drastic increase in the numbers of patients seeking orthodontic treatment. The most important motivation for orthodontic treatment is to achieve an improvement in appearance, masticatory efficiency with associated improvement in self-esteem and confidence.[1] During active orthodontic treatment important consideration has to be given to the health of teeth and the periodontium, because ultimately the efficiency of orthodontic treatment depends upon the response of the periodontal tissues to orthodontic intervention. Orthodontic adhesive remaining around the bracket base can be a strong predisposing factor for plaque retention because the rough adhesive surface provides an ideal site for the rapid attachment and growth of oral microorganisms.[3],[4]

A mouth rinse is a chemotherapeutic agent used as an effective home care remedy to enhance oral hygiene and prevent dental caries by targeting the cariogenic bacteria. Mouthwashes can inhibit dental plaque, and are widely used to maintain oral hygiene. Dental plaque harbors not only gram-positive streptococci but also gram-negative anaerobic bacteria aggregation.[4],[5]

Nowadays, in most studies on mouthwashes, chlorhexidine is used as a positive control to compare the efficacy of other products, since it is believed that chlorhexidine is a gold standard.[6] The incidence of side effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth discourage patients to use this mouthwash.[7] Because of these side effects of chlorhexidine mouthwash, the search for an effective antimicrobial mouthwash with less side effects still continues.[8]

Recently, the use of herbal mouthwashes is increasing. It has been shown that using herbal medicine or its extract would support periodontal health, and reduces the accumulation of microbial plaques, and bleeding during brushing and controls gingivitis and periodontal diseases.[9],[10] Similarly Freshclor mouth rinse possess as an excellent bactericide, fungicide, and antimicrobial agent. The active ingredient in Freshclor is a stabilized molecular form of chlorine dioxide.[11] Herbal mouth rinse and Freshclor doesn't cause brown discoloration of teeth and other oral surface; it doesn’t alter the taste. So in this study we are trying to find out a mouthwash which has maximum antibacterial activity and least number of side effects among above mentioned mouthwashes.

Material and method

Thirty patients undergoing fixed ortho dontic treatment at Department of Orthodontics and Dento facial Ortho pedics, were selected for the study.

Inclusion criteria

- Subjects who were between 12-20years of age group with complete permanent dentition till second permanent molars undergoing orthodontic treatment (extraction or non-extraction case) at least three months prior to start of the study,
- Subjects with generally good health and had no positive history of any systemic disease or antibiotic medication that would influence the presence or count of the oral microorganisms.
- Subjects with normal gingiva, without inflammation, bleeding, discoloration with fairly good oral hygiene.

- Subjects with no evidence of decalcification of teeth and no caries found in any teeth.

Exclusion criteria

- Subjects who had antibiotic or antibacterial medication or mouth rinse for 10 consecutive days in last three months.
- Subjects who reported sensitivity to any mouth rinse.
- Subjects who had some systemic medical problem or disease.
- Subjects who had severe gingival inflammation.
- Subjects who were in mixed dentition stage.

Each subject used oral rinses with three different mouthwashes (figure-1) and distilled water. Accordingly, four different groups were identified having 30 samples each.

Group A: Those samples which were collected after oral rinses with distilled water. This served as a control group.

Group B: Those samples which were collected after oral rinses with 0.2 % chlorhexidine Gluconate (Clohex – Dr. Reddy's lab, India) twice daily undiluted as per manufacturer's directions.

Group C: Those samples which were collected after oral rinses with 0.1% stabilized chlorine dioxide (Freshclor - Group Pharmaceuticals, India) twice daily undiluted as per manufacturer's directions**.**

Group D: Those **s**amples which were collected after oral rinses with herbal mouthwash (Hiora - Himalaya Pharmaceuticals, India) twice daily undiluted as per manufacturer's directions.

Fig 1: Mouthwashes used in the study. From left to right- Chlorhexidine IP (CLOHEX, Dr. REDDY'S laboratory, India), B. Stabilized Chlorine Dioxide- 0.1% (Freshclor, Group Pharmaceuticals, India), C. Herbal mouthwash (Hiora, Himalaya Pharmaceuticals, India)

The samples in the form of ligating elastic modules (Figure-2) used intraorally for three weeks were collected from each subject as per the time interval stated below. Four samples were totally collected for each subject.

Fig 2: Site of module sample collection

1. Sample 1(Gp A): First sample was collected three weeks after bonding of brackets. During this period the subjects were advised to use distilled water as mouth rinse to prevent changes in microflora.

2. Sample 2 (Gp B): After collection of first sample, subjects were prescribed to use chlorhexidine mouth rinses undiluted twice daily as directed by manufacturer for three weeks and the second sample was collected after this period of three weeks.

After collecting the second sample subjects were prescribed to use oral rinses with distilled water twice daily for three weeks to nullify effect of previous mouth rinse.

3. Sample 3 (Gp C): After collection of second sample, subjects were prescribed to use stabilized chlorine dioxide (Freshclor - Group Pharmaceuticals, India) mouth rinse twice daily as directed by manufacturer for three weeks and the third sample was collected after this period of three weeks.

After collecting the third sample the subjects were prescribed to use oral rinses with distilled water twice daily for three weeks to nullify effect of previous mouth rinse.

4. Sample 4 (Gp D): After collection of third sample, subjects were prescribed to use herbal mouthwash (Hiora - Himalaya Pharmaceuticals, India) mouth rinse twice daily as directed by manufacturer for three weeks and the third sample was collected after this period of three weeks.

Four samples thus collected in the form of modules coated with plaque from each patient were transferred to a sterile plastic container, properly labeled by subject name and group name, containing one ml of normal saline (N.S).

In the laboratory, the labeled vials containing the module samples were placed on a vortex mixer (Remi equipments, India) for 2 minutes in order to disperse the material uniformly. A 0.1ml of each sample was diluted to 1:100 dilutions with 1ml of saline water (0.9% NaCl) for ease of counting of colonies later on. Mutans Sanguis Agar Media (Hi-Media Laboratories Pvt. Ltd. Mumbai) India) was chosen for isolation and culture of the organism.

Autoclave procedure

Petri dish and Flask containing media were autoclaved at 121^0 C temperatures at 15 lbs. pressure for 30 minutes. Pressure was released from autoclave for 15 minutes till it came to zero lbs. Flask containing media and Petri dish were taken to the laminar air flow (Fig 3) where the temperature of media was lowered up to 50^0 C.

Fig. 3: Laminar Air Flow (NAVYUG-UDYOG NU-157, Haryana, India)

Incubation procedure

The media was poured into the Petri plate and allowed to solidify. A 0.1 ml of sample solution was taken using micropipette and spreaded onto the medium surface using sterilized glass spreader. Plates were placed into the incubator at anaerobic conditions for 24 hours at 370 C (Fig 4).

Fig 4: Incubator (SCIENTECH IN – 308, New Delhi, India)

Counting of viable colonies of Streptococcus mutans

After 24 hours of incubation each plate was placed under digital colony meter (Fig 5) for counting the viable colonies (fig 6). The number of Colony Forming Units (C. F. U's) i.e., the number of viable microorganisms per 2 milliliters of the sample were calculated.

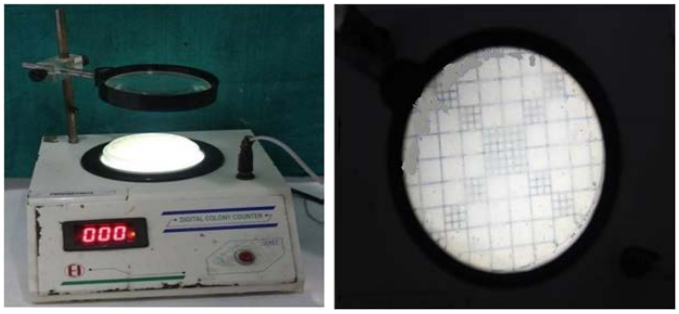

Fig 5: Digital colony counter (Mv tex Science Industries, Haryana, India.

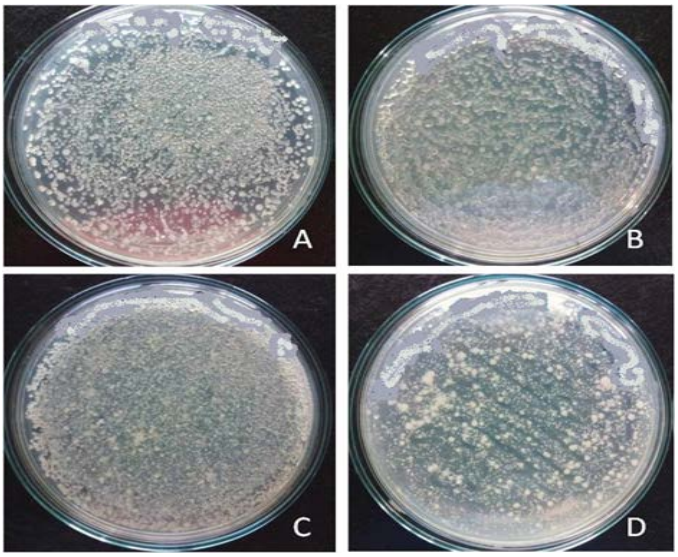

Fig 6: Streptococcus Mutans viable colonies visible in all four samples. A-Sample 1, B-Sample 2, C-Sample 3, D-Sample 4.

Statistical analysis

was done using Statistical Package of Social Science (SPSS Version 20; Chicago Inc, USA). Data comparison was done by applying Kruskal-Wallis Test and Mann Whitney 'U' Test to find out the statistical significance of the comparisons.

Results

This present study was carried out to assess comparative evaluation of the antibacterial effects of three commonly prescribed mouth rinses 0.2% chlorhexidine gluconate, 0.1% stabilized chlorine dioxide and herbal mouth wash on Streptococcus Mutans in orthodontic patients, where oral rinses with distilled water being used as control group. Accordingly, four groups were identified. The data obtained was compiled and tabulated and was subjected to statistical analysis. A master table was prepared and the total data was subdivided and distributed meaningfully and presented as individual tables along with graphs.

Table 1: shows CFU/ml count of all four groups for all 30 subjects used for the present study.

S.NO	TEST SAMPLE	CONTROL CFU/ml	CLOHEX CFU/ml	FRESHCLOR CFU/ml	HIORA CFU/ml
1	Test sample 1	241	91	96	104
2	Test sample 2	244	133	182	119
3	Test sample 3	223	93	109	112
4	Test sample 4	229	129	141	116
5	Test sample 5	250	117	134	107
6	Test sample 6	243	113	152	100
7	Test sample 7	217	117	156	119
8	Test sample 8	250	97	171	115
9	Test sample 9	239	118	135	107
10	Test sample 10	212	106	123	117
11	Test sample 11	199	196	221	196
12	Test sample 12	198	113	242	232
13	Test sample 13	195	211	203	252
14	Test sample 14	168	143	215	101
15	Test sample 15	189	171	210	122
16	Test sample 16	268	157	229	209
17	Test sample 17	278	136	216	139
18	Test sample 18	274	125	219	207
19	Test sample 19	282	107	239	215
20	Test sample 20	199	138	237	219
21	Test sample 21	264	123	136	131
22	Test sample 22	246	190	119	162
23	Test sample 23	193	176	192	151
24	Test sample 24	222	112	196	187
25	Test sample 25	262	103	154	147
26	Test sample 26	196	129	152	141
27	Test sample 27	237	134	159	149
28	Test sample 28	266	113	143	139
29	Test sample 29	213	127	173	162
30	Test sample 30	272	129	149	140

Table 2: present comparison of mean CFU/ml (S. Mutans) among Clohex, Freshclor, Hiora mouth rinse and controls (distilled water rinses) in orthodontic patients. Mean CFU/ml (S. Mutans) was found minimum in Clohex mouth rinse i.e. 131.57±30.42 and highest in Freshclor mouth rinse i.e. 173.43±42.19. It

was 150.57±43.84 in Hiora mouth rinse. Statistically highly significant difference was found between all three mouth rinses and control in respect to Mean CFU/ml (P=0.001).

		Mean CFU/ml			
Groups	N	MEAN	S.D	Median	Range
CLOHEX	30	131.57	30.42	126.00	91-211
FRESHCLOR	30	173.43	42.19	165.00	96-242
HIORA	30	150.57	43.84	139.50	100-252
CONTROL	30	232.30	31.21	238.00	168-282
TOTAL	120	171.97	53.01	160.50	91-282
Kruskal-Wallis Test (X^2 Value)		58.477			
Significance 'P' Value		0.001(HS)			

Table 2: Comparison of Mean CFU/ml (S. Mutans) among Clohex, Freshclor, Hiora mouth rinse and Controls (distilled water rinse) in orthodontic patients.

When comparing between Clohex and Freshclor mouth rinse in orthodontic patients, Mean CFU/ml in Clohex mouth rinse (131.57± 30.42) is less as compared to that of Freshclor mouth rinse (173.43±42.19), which is statistically highly significant (P=0.001)

When comparing between Clohex and Hiora mouth rinse in orthodontic patients, Mean CFU/ml in Clohex mouth rinse (131.57± 30.42) is less as compared to that of Hiora mouth rinse (150.57±43.84), which is statistically not significant (P=0.92)

When comparing between Clohex and Control (distilled water) mouth rinse in orthodontic patients, Mean CFU/ml in Clohex mouth rinse (131.57± 30.42) is less as compared to that of distilled water mouth rinse (232.30±31.21), which is statistically highly significant (P=0.001).

When comparing between Hiora and Freshclor mouth rinse in orthodontic patients, Mean CFU/ml in Hiora mouth rinse (150.57±43.84) is less as compared to that of Freshclor mouth rinse (173.43±42.19), which is statistically significant (P=0.022).

When comparing between Freshclor and Control (distilled water) mouth rinse in orthodontic patients, Mean CFU/ml in Freshclor mouth rinse (173.43±42.19) is less as compared to that of distilled water mouth rinse (232.30±31.21), which is statistically highly significant (P=0.001).

When comparing between Hiora mouth rinse and Control (distilled water) mouth rinse in orthodontic patients, Mean CFU/ml in Hiora mouth rinse (150.57±43.84) is less as compared to that of distilled water mouth rinse (232.30±31.21), which is statistically highly significant (P=0.001).

Discussion

This present study was carried out to assess comparative evaluation of the antibacterial effects of three commonly prescribed mouth rinses 0.2% chlorhexidine gluconate, 0.1% stabilized chlorine dioxide and herbal mouth wash on streptococcus mutans in orthodontic patients, where oral rinses with distilled water being used as control group. Among the various mouth rinses, the most persistent reduction of S. mutans has been achieved by chlorhexidine mouth rinses.[7], [13-17] The application of Clohex induced a significant reduction of S. mutans in saliva over a one-month period and a reduction in the proportion of S. mutans in the plaque adjacent to brackets.[3] Chlorhexidine gluconate is one of the most widely used broad spectrum antibacterial or antiseptic agents in dentistry. The prevalence of bacteremia found with chlorhexidine application was less than the prevalence obtained without chlorhexidine.[13] Some of the side effects of using chlorhexidine that limit its widespread acceptance include brown staining of the teeth, an increase in calculus deposition, and the

difficulty in completely masking its taste when used as a rinse.[17]

Recently, the use of herbal mouth rinse is increasing. Hiora is a phytopharmaceutical dental preparation used as refreshing oral rinses formulated to maintain and enhance oral health and hygiene, by providing antiseptic and antimicrobial activities.[7] The clinical efficiency and safety of Hiora mouth rinse in comparison with Chlorhexidine mouth rinse in improving oral health in patients undergoing dental procedures needed proper evaluation. Freshclor mouth rinse possesses excellent bactericide, fungicide, and antimicrobial properties. The active ingredient in Freshclor is a stabilized molecular form of chlorine dioxide. Chlorine dioxide has been used in water purification for over fifty years and has been certified safe by the U.S. Environmental Protection Agency (EPA).[10] This ingredient is registered with the Environmental Protection Agency as an excellent bactericide, fungicide, and antimicrobial agent. Freshclor doesn't cause brown discoloration of teeth and other oral surface; it doesn't alter the taste and doesn't interfere with fibroblasts which are associated with healing whereas chlorhexidine interferes with fibroblast activity.[11]

In the present study counting of streptococcus mutans colonies from the samples which were collected from the patients under orthodontic treatment was done. Though all three antibacterial mouth washes tested caused reduction of S. mutans counts, there were significant differences in S. mutans colonization following use of Clohex, Freshclor, Hiora mouth rinses. Comparison of Mean CFU/ml (S. Mutans) among Clohex, Freshclor, Hiora mouth rinses and control (distilled water rinses) groups was done. Mean CFU/ml (S. Mutans) was found minimum in Clohex mouth rinse 131.57±30.42 and highest in Freshclor mouth rinse 173.43±42.19. It was 150.57±43.84 in Hiora mouth rinse, while control group had highest count 232.30±31.21. Statistically highly significant difference was found between all three mouth rinses and control in respect to Mean CFU/ml. ($P<0.001$)

The mean value of CFU/ml in Clohex and Hiora groups was almost similar while comparison of the mean value between Clohex and Freshclor showed that Clohex was significantly more effective than Freshclor. Also, in the comparison between Hiora and Freshclor, Hiora was significantly more effective than Freshclor. While comparing these three groups with the control group (distilled water), the difference in the mean values were highly significant for all three groups. Control group showed the maximum number of streptococci mutans colonies as compared with three different mouth rinses. Our results corroborate with the findings of other previous studies done by Enita et al [17], Aneja KR et al[15], Sari et al[13], Fard et al[16], where chlorhexidine mouthwash was found to be more effective on streptococcus mutans in decreasing the number of CFU/ml colonies as compared to other mouth washes. Our results also corroborate with the study of Salehi P et al [9] who has compared Persica (Herbal mouthwash) with chlorhexidine mouthwash. Persica mouthwash was found to have potent antibacterial effects but less than Clohex mouthwash. In present study also, Hiora mouthwash (herbal mouthwash) showed good antibacterial effects on S. mutans but less than that of Clohex group.

From the results obtained, it is evident that Clohex mouthwash was most effective followed by Hiora and Freshclor mouthwash in controlling growth of S. mutans colonies. Efficiency of Hiora mouthwash was slightly lower than that of Clohex mouthwash though former was more refreshing and pleasant for patients. Most subjects

in our study reported altered taste of mouth, and burning sensation of mouth, with brown staining of teeth with Clohex mouth wash. These untoward effects of Chlorhexidine gluconate mouth wash indicate that Hiora herbal mouthwash is more preferable over Chlorhexidine when long term use is required. Freshclor mouthwash having stabilized chlorine dioxide is also a good option with good taste and no side effects, but its low antibacterial efficacy compared to Hiora and Clohex limits its use.

This study is a first study to compare efficiency of Clohex (chlorhexidine gluconate) with Hiora (herbal) and Freshclor (stabilized chlorine dioxide) mouthwashes. There are no previous studies comparing the effectiveness of stabilized chlorine dioxide mouthwash to test their antibacterial efficiency, except the study by Salehi et al [9] who has used a Persica a herbal mouthwash and compared it with chlorhexidine mouthwash. So there is a huge scope of further studies needs to be done in this field using herbal and other mouthwashes to find their efficacy and to confirm the results obtained by this study.

Conclusion

The present study were drawn based on the findings of following conclusions that Clohex and Hiora mouth rinses were extremely effective in reducing S. mutans colonies levels. Chlorhexidine was more effective against S. mutans as it showed lesser CFU/ml value than Hiora mouth wash. In the Freshclor group, the streptococcus mutans colonies were comparatively higher than in the Hiora and Clohex mouth rinses groups. Though chlorhexidine showed highest control of streptococcus mutans colony growth the subjects reported bitter unpleasant taste and more brownish staining of teeth with chlorhexidine mouth washes. Freshclor was more acceptable for the subjects being refreshing with pleasant taste. Hence though very effective against streptococcus mutans, the orthodontic patients who are prescribed oral rinses with chlorhexidine mouth washes should be informed of these side effects. As expected, distilled water rinses were not effective in controlling streptococcus mutans count as this group had highest CFU/ml values. This continues need for use of antibacterial mouth washes during orthodontic treatment to control growth of streptococcus mutans.

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