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To Determine and Compare the Antibacterial Efficacy of Hiora Mouthwash with 0.2% Chlorhexidine Gluconate in Orthodontic Patients

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KEYWORDS
Hiora mouthwash, Chlorhexidine mouthwash, bacteria, Orthodontic patients.

ABSTRACT:

Aim and objectives: To determine and compare the antibacterial efficacy of Hiora mouthwash with 0.2% chlorhexidine gluconate in orthodontic patients after bonding at different time intervals for a period of 3 months of each patient.

Materials and Methods: A total of 60 orthodontic patients were selected for the study. Auxillaries used were test tubes, periodontal probe (Williams probe), Wilkins probe, mouth mirror, cheek retractor and icebox. Saliva sample was collected at base line, 1st day of bonding (T0), 30th day (T1) and 90th day (T2) of 3 groups which were Group A (control group),Group B (Chlorhexidine group) and Group C (Hiora Group) for the microbial count of mouth and gingival assessment was also done at the same time points. Microbial counts were done by conventional culture method. Paired t-test, t-test, chi square test followed by post hoc Tukey test were done to compare the groups statistically.

Results: The study revealed that the microbial count of mouth increases after bonding, 0.2% Chlorhexidine gluconate mouthwash and Hiora mouthwash reduced the microbial count and gingival inflammation significantly at T2.

Conclusion: 0.2% Chlorhexidine gluconate mouthwash is more effective in terms of reduction of microbial count and gingival inflammation.

Introduction

The malocclusions are among the main buccal health problems all over the world, together with dental cavity and periodontal disease.[1] Orthodontic treatment aims to adjust the position of teeth to the right tooth curve, improve efficiency of chewing function, face harmony, oral health, dentofacial aesthetics, and tooth position stability. The Orthodontic treatment usually takes 2–3 years[2] and its difficult to maintain oral hygiene during this period due to the difficulties in brushing due to malocclusion and fixed orthodontic appliance which further increase the accumulation of microbial plaque and decrease salivary flow.[3]

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Dental Plaque is a biofilm with layers of microorganisms contained in a matrix that forms on the oral surface.[4] Shirozaki et al conducted a study to analyze clinical, microbiological, and immunological periodontal parameters with patients in corrective orthodontic treatment. They found out that, corrective orthodontic treatment caused clinical periodontal alterations regarding biofilm accumulation and gingival bleeding, with alteration of periodontopathogens.[5] Streptococci are the primary inhabitants of the oral cavity which are attained after birth and they play a crucial role in the congregation of the oral microbiota. However, they have a remarkable ability to metabolize carbohydrates via fermentation thereby generating acids as by products and this extreme acidification of the oral environment by aciduric species is associated with the progress of dental caries. Streptococcus mutans, also has a significant function in plaque formation and accumulation. Hameed et al concluded that fixed orthodontic appliances lead to an increase in the volume and number of cariogenic streptococci in dental plaque.[3] Staphylococcus aureus is another bacteria which adhere and form biofilms on materials and cells and it is detected in high frequencies when the immune response is reduced. Merghni et al conducted a study and found out a high potential of adhesion to biotic and abiotic surfaces by opportunistic Staphylococcus aureus strains in orthodontic patients. They concluded that S. aureus is a redoubtable pathogen associated with orthodontic material infections.[6] Erverdi et al referred from his study that samples from 7.5% of patients after orthodontic banding showed bacteremia, and Streptococcus sanguis and Streptococcus mitis were isolated.[7] The primary causative factor in the development of gingivitis during orthodontic treatment is the insufficient removal of supragingival plaque.[3] Mechanical tooth cleaning is very important for patients with fixed orthodontic appliances. However few orthodontic patients have difficulties in maintaining plaque control by mechanical means alone.[8] Several chemical antiplaque agents are available commercially, and they can be delivered in the form of mouthwash, dentifrices, chewing gums, and gel.[3] The usage of a mouthwash is the easiest and convenient to perform, it could be a handy clinical adjunct for reducing the bacterial plaque accumulation.[3] Mouth rinses have the ability to deliver the therapeutic effect all over the tooth surface including interproximal areas in which even toothpastes are not much effective.[9] Lundstrom et al studied the development of new carious lesions during Orthodontic treatment with fixed appliances in patients with high levels of caries inducing oral microflora (S. mutans) in saliva prior to treatment with and without chlorhexidine mouthwash. A difference in caries incidence was recorded between the groups primarily on buccal surfaces of bonded teeth; but this was not found to be statistically significant.[10] Chlorhexidine gluconateis considered the most effective antiplaque mouth rinse and in preventing dental caries. Its mechanism of action is mainly based on the rupture of bacterial cell walls which subsequently causes precipitation of the cell cytoplasmic contents.[3] Chlorhexidine is an agent that is frequently used against S mutans. It is commercially available in the forms of mouth rinse, gel, and varnish.[8] Therefore the main aim of the present study is to investigate and compare the effect of 0.2% chlorhexidine gluconate mouth rinse and herbal mouthwash on Streptococcus mutans, Streptococcus sanguinis and Staphylococcus aureus in orthodontic patients with fixed appliances.

Material and methods

The research was carried out in the Department of Orthodontics and Dentofacial Orthopaedics of Surendera Dental College and Research Institute, located in Sri Ganganagar, Rajasthan. Ethical clearance was obtained from Institutional Ethical Committee (SDCRI/IEC/22/04). 60 Participants were included in this study. Total of 20 samples in each group. All subjects were in the age group of 12-30 years. Patients with no history of previous orthodontic treatment, Patients with no systemic disease history, Patients who had not been on antibiotics therapy during last 3 months before the commencement of study, No known hypersensitivity to 0.2% chlorhexidine gluconate and Hiora mouthwash and No anterior composites present were included in this study.

Methodology

60 patients were divided into 3 groups. Two different types of mouthwashes were used (Fig.1). Distilled water was used for Control group. The patients were allocated to three main groups by using Table of Randomization Method. Group A (Control=20), Group B (Chlorhexidine mouthwash=20) and Group C (Hiora Mouthwash=20). All patients have used the self-

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dispensing mouthwash. After subjecting the patient to initial oral prophylaxis, the clinical parameters for Gingival Index (GI) were recorded. Gingivitis assessment was done using the Gingival Index (Loe and Silness, 1963) to record the gingival health status. Gingival indices were recorded with the help of mouth mirror, Williams graduated periodontal probe and wilkins explorer (Fig. 2). The severity of gingivitis was scored on selected index teeth such as - 16,12,24,36,32,44. The teeth and gingiva were dried lightly with a blast of air and cotton rolls. Scoring criteria ranged from 0 to 3 and assessed area were disto-facial, facial, mesiofacial and lingual surfaces. (Fig. 3-6)

Gingival values were recorded at three different time intervals:

- At baseline, before fixed orthodontic treatment (T0)
- 1st month of orthodontic treatment (T1)
- 3rd month of orthodontic treatment (T2)

Saliva samples were collected from the patient by instructing them to chew a piece of paraffin for a duration of 5 minutes, until a total volume of 3ml of saliva was obtained. The saliva sample was stored at low temperatures (0° C) by placing it in ice prior to its use followed by -20° C in laboratory. A total of 20 patients from Group A were administered fixed orthodontic treatment and provided with instructions to clean their mouths twice daily with distilled water. During the first consultation, the dental professional performed oral prophylaxis, followed by the assessment of Gingival Index (GI). Additionally, a saliva sample was collected to gather data for microbiological examination. GI and saliva sample was taken on the second visit on the 30th day and the third appointment on the 90th day. A total of 20 participants from Group B were provided with instructions to rinse their mouths twice daily using 10ml of mouthwash containing 0.2% Chlorhexidine Gluconate for a duration of 2 minutes. During the first session, the patient had oral prophylaxis, which was followed by the assessment of Gingival Index (GI). Additionally, a saliva sample was collected to facilitate microbiological analysis and data collecting. BOP and saliva sample was taken on the second visit on the 30th day and again during the third appointment on the 90th day.A total of 20 patients from Group C were directed to do oral rinsing twice daily using 15ml of Herbal mouthwash for a duration of 30 seconds. During the first session, the dental professional performed oral prophylaxis, followed by the assessment of Gingival Index (GI). Additionally, a saliva sample was collected to facilitate microbiological analysis and data collecting. BOP and saliva sample was taken on the second visit on the 30th day and the third appointment on the 90th day. The amounts of Streptococcus mutans, Streptococcus sanguinis and Staphylococcus aureus in saliva samples were assessed at three distinct time points, namely T0, T1, and T2 with the help of conventional culture method at J. Sidana Diagnostic Laboratory. Culture media or growth media was used for the growth of the bacteria. Two types of agar were used such as nutrient agar and blood agar. For Streptococcus mutans and Streptococcus sanguinis, blood agar was used and for Staphylococcus aureus nutrient agar was used (Fig. 7-8).

Fig. 1: Different mouthwashes dispensed to the patients

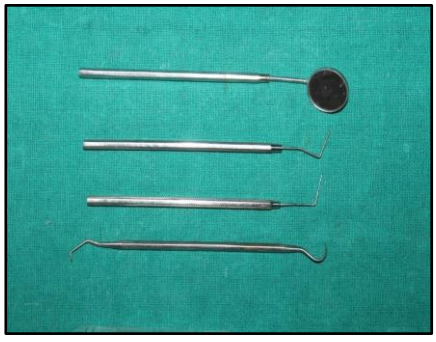

Fig. 2: Armamentarium

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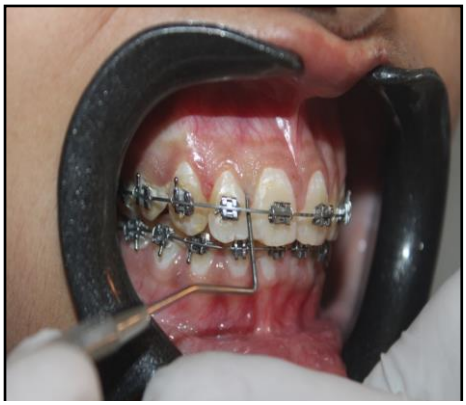

Fig. 3: Gingival assessment on mesio-labial surface of lateral incisor

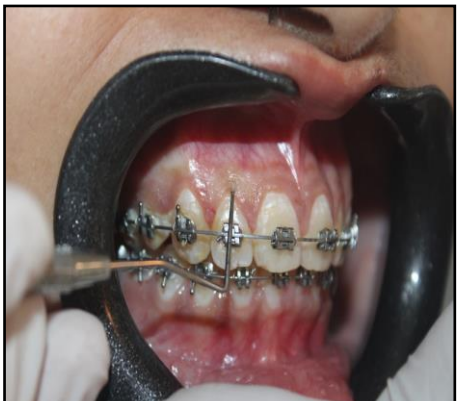

Fig. 4: Gingival assessment on labial surface of lateral incisor

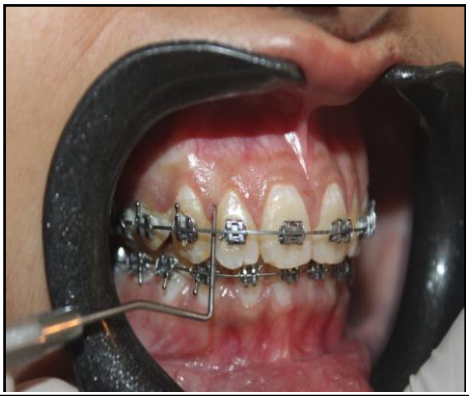

Fig. 5: Gingival assessment on disto-labial surface of lateral incisor

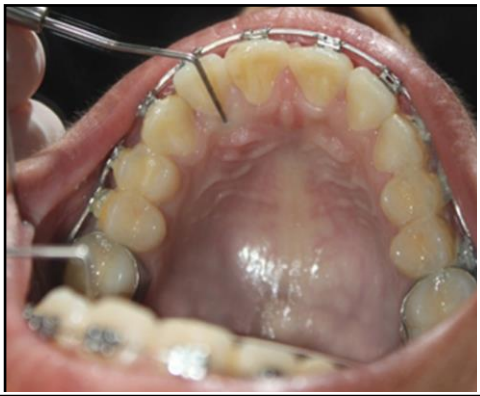

Fig. 6: Gingival assessment on palatal surface of lateral incisor

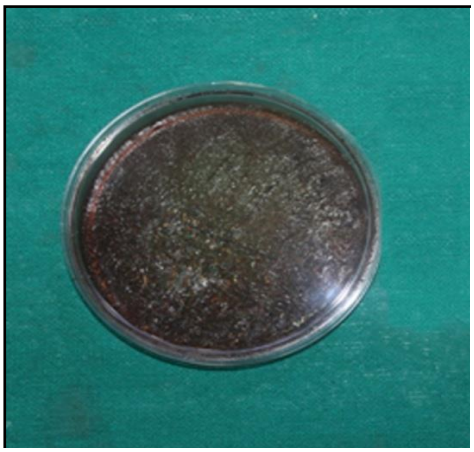

Fig. 7: Blood Agar for Streptococcus mutans & Streptococcus sanguinis

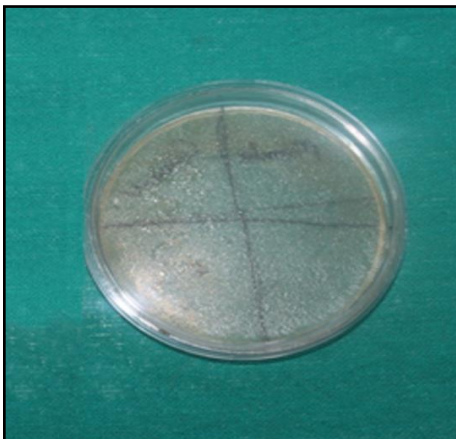

Fig. 8: Nutrient Agar for Staphylococcus aureus

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Statistical analysis

Statistical analysis was done using Statistical Package of Social Science (SPSS Version 21.0; Chicago Inc., USA). Data comparison was done by applying specific statistical tests to find out the statistical significance of the comparisons. Significance level was fixed at $P < 0.05$.

Results

Total 60 patients of age group between 12-30 with fixed orthodontic appliances were selected from OPD of Department of Orthodontics and Dentofacial Orthopaedics, Surendera Dental College and Research Institute, Sri Ganganagar for the study. The patients were allocated to three main groups, Group A (Control group), Group B (Chlorhexidine group) and Group C (Hiora Group).

Gingival Index (GI) was recorded at three different time intervals and saliva was collected also at three time intervals at baseline, before fixed orthodontic treatment (T0), 1st month of orthodontic treatment (T1), 3rd month of orthodontic treatment (T2). The amounts of Streptococcus mutans, Streptococcus sanguinis and Staphylococcus aureus in saliva samples were assessed at these distinct time points, namely T0, T1, and T2 with the help of conventional culture method.

Table 1 showed the mean values of various bacteria at different time intervals in group A(control group). All the bacteria showed statistically significant increase in number with time. Maximum value(346.5 CFU(log)/ml) was found with respect to Streptococcus mutans and minimum value (143.9 CFU(log)/ml)was obtained for Streptococcus sanguinis at T2. (Table 1)

Table 2 and 3 showed the mean values of various bacteria at different time intervals in group B and C respectively. All the bacteria showed statistically significant decrease in number with time. Maximum value was found with respect to Streptococcus mutans and minimum value was obtained for Streptococcus sanguinis at T2. (Table 2, table 3)

Post hoc Tukey analysis in table 4 showed comparison of various bacteria among the various study groups and a statistically significant difference ($p<0.01$)was obtained when group A was compared with either Group B or Group C. On comparison of Group B and Group C, statistically significant difference ($p<0.01$) was obtained at T2. (Table 4, Table 5, Table 6)

Table 7 showed comparison of gingival values among the study groups at different time intervals. Gingival values showed an increase in number with time in Group A and decrease in number with time in Group B and Group C .Post hoc Tukey analysis in table 7 showed comparison among the study groups and a statistically significant difference ($p<0.01$) was obtained when Group A was compared with either Group B or Group C. On comparison of Group B and Group C, statistically significant difference ($p<0.01$) was obtained at T1 and T2. (Table 7)

Table 1: Group A showing the mean values of various bacteria at different time intervals

Interval	Streptococcus mutans		Streptococcus sanguinis		Staphylococcus aureus	
	Mean	SD	Mean	SD	Mean	SD
T0	79.4	20.8	30.2	7.03	42.8	6.4
T1	302.7	41.3	105.6	12.55	91.3	11.6
T2	346.5	51.9	143.9	24.7	185.4	19.2
p value						
T0 vs T1	<0.01*		<0.01*		<0.01*	
T0 vs T2	<0.01*		<0.01*		<0.01*	
T1 vs T2	<0.01*		<0.01*		<0.01*	

Paired t test, *: statistically significant:p<0.01*

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Table 2: Group B showing the mean values of various bacteria at different time intervals

Interval	Streptococcus mutans		Streptococcus sanguinis		Staphylococcus aureus	
	Mean	SD	Mean	SD	Mean	SD
T0	84.2	19.01	29.7	6.95	41.06	5.87
T1	67.5	16.08	23.3	8.14	32.6	7.21
T2	43.8	15.05	14.4	5.47	18.1	4.62
p value						
T0 vs T1	<0.01*		<0.01*		<0.01*	
T0 vs T2	<0.01*		<0.01*		<0.01*	
T1 vs T2	<0.01*		<0.01*		<0.01*	

Paired t test, *: statistically significant:p<0.01*

Table 3: Group C showing the mean values of various bacteria at different time intervals

Interval	Streptococcus mutans		Streptococcus sanguinis		Staphylococcus aureus	
	Mean	SD	Mean	SD	Mean	SD
T0	86.5	21.31	32	9.25	43.36	8.17
T1	71.6	20.18	27.4	12.24	36.7	11.31
T2	48.3	19.55	18.9	9.97	22.6	9.12
p value						
T0 vs T1	<0.01*		<0.01*		<0.01*	
T0 vs T2	<0.01*		<0.01*		<0.01*	
T1 vs T2	<0.01*		<0.01*		<0.01*	

Paired t test, *: statistically significant :p<0.01*

Table 4: Comparison of Streptococcus mutans among the study groups

Group	T0		T1		T2	
	Mean	SD	Mean	SD	Mean	SD
Group A	79.4	20.8	302.7	41.3	346.5	51.9
Group B	84.2	19.01	67.5	16.08	43.8	15.05
Group C	86.5	21.31	71.6	20.18	48.3	19.55
p value$^{\infty}$						
Group A vs Group B	0.69		<0.01*		<0.01*	
Group A vs Group C	0.61		<0.01*		<0.01*	
Group B vs Group C	0.84		0.17		0.045*	

$^{\infty}$: t test, *: statistically significant :p<0.01*

Table 5: Comparison of Streptococcus sanguinis among the study groups

Group	T0		T1		T2	
	Mean	SD	Mean	SD	Mean	SD
Group A	30.2	7.03	105.6	12.55	143.9	24.7
Group B	29.7	6.95	23.3	8.14	14.4	5.47
Group C	32	9.25	27.4	12.24	18.9	9.97
p value$^{\infty}$						

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Group Avs Group B	0.79	<0.01*	<0.01*
Group Avs Group C	0.70	<0.01*	<0.01*
Group B vs Group C	0.65	0.27	0.041*

∞: t test,*: statistically significant :p<0.01*

Table 6: Comparison of Staphylococcus aureus among the study groups

Group	T0		T1		T2	
	Mean	SD	Mean	SD	Mean	SD
Group A	42.8	6.4	91.3	11.6	185.4	19.2
Group B	41.06	5.87	32.6	7.21	18.1	4.62
Group C	43.36	8.17	36.7	11.31	22.6	9.12
p value∞						
Group Avs Group B	0.76		<0.01*		<0.01*	
Group Avs Group C	0.63		<0.01*		<0.01*	
Group B vs Group C	0.54		0.23		0.09	

∞: t test, *: statistically significant :p<0.01*

Table 7: Gingival Index comparison among the study groups

Group	T0	T1	T2
Group A	0.60	1.20	1.65
Group B	0.65	0.35	0.30
Group C	0.60	0.50	0.45
p valueβ			
Group A vs Group B	0.65	<0.01*	<0.01*
Group A vs Group C	0.59	0.007*	<0.01*
Group B vs Group C	0.77	0.032*	0.038*

β: chi square, *: statistically significant

Discussion

Fixed orthodontic appliances are considered to be a clinical risk factor in terms of enamel integrity because of plaque accumulation around the bracket base.[11] Sari et al evaluated and analyzed the changes and levels of S mutans and lactobacilli in saliva samples and showed that S mutans and lactobacilli levels were significantly increased after bonding fixed appliances. They concluded that difficulty in brushing during orthodontic treatment induces periodontal problems with deterioration of the ecologic balance of the oral flora.[12] Khobragede et al mentioned that plaque control should be an indispensable part of the daily chores of every individual as the onset of dental diseases can be primarily prevented by regular and meticulous plaque removal. Toothbrushing, when accomplished properly, results in effective plaque control. However, whenever it is accompanied by chemical plaque control measures, it gives a synergistic effect. Therefore, adjunctive chemical plaque control methods such as the use of

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mouthwash have been suggested as an additional therapeutic strategy to augment but definitely not to replace mechanical plaque control.[4] In a study report by Shilpa M et al, it was concluded that manual brushing does not only improve plaque control and gingival health on orthodontic patients, however, manual brushing along with chlorehexidine mouthwash reduces bleeding scores, improves plaque control and gingival health. Hence, a reduction in gingivitis is seen in patients using manual brushing combined with chlorhexidine mouthwash.[13]

However, chlorhexidine mouthwashes have side effects such as tooth discolouration and sense of burning or dryness in the mouth and metallic taste on prolonged use, so it cannot be prescribed for longer duration.[14] It was reported in a study by Gurgan and Zaim, chlorhexidine mouthwash caused more irritation to oral mucosa and the frequency of burning sensation and taste disturbance were high.[15]

Few studies have compared the antimicrobial efficacy of various herbal mouthwashes with Chlorhexidine mouthwash, but none of the study has compared Hiora with Chlorhexidine from microbial aspect. So in this study chlorhexidine and hiora mouthwash were prescribed to the patients to maintain gingival health during orthodontic treatment and to assess the efficacy of 2 mouthwashes, microbial evaluation was done by taking saliva samples and clinical evaluation by gingival index.

Total 60 patients of age group between 12-30 with fixed orthodontic appliances were selected from OPD of the Department. Inclusion criteria included patients who had not been on antibiotics therapy during last 3 months before the commencement of study with satisfactory oral hygiene.

Saliva samples were taken at T0 (day of bonding), T1 (30^{th} day) and T2 (90^{th} day) of Group A, Group B and Group C to observe the significant microbiological difference. After the saliva samples were taken, they were sent for microbial examination by conventional culture method.

Weinstein M P mentioned that conventional manual systems and media are available from many commercial sources. Conventional manual systems are flexible and require no purchase of expensive instruments.[16] In this study, culture media or growth media was used for the growth of Streptococcus mutans, Streptococcus sanguinis (Blood agar) and Staphylococcus aureus (Nutrient agar). Incubation was done for 24 hour at 35 degree to 37 degree temperature for the growth of bacteria. Then primary and secondary identification were done. Biochemical reaction for secondary identification was done to understand the specific species to get the specific colony and then colony counting was done.

The results of the study revealed that bacterial count increased significantly between T0-T1 and T1-T2 in group A. These results are in the favour of Gastel J V and Ristic M et al who mentioned that the placement of fixed orthodontic appliances had a significant impact on microbial and clinical variables. The changes occurred because wire insertion caused difficulties in proximal cleaning which may transitionally increase the values of all periodontal indices and stimulate the growth of periodonto-pathogenic bacteria.[17,18]

The results also displayed that GI values increased from T0 to T2 in Group A. This is in accordance with Gong Y et al who mentioned that periodontal pathogens might have a relationship with the initiation and development of orthodontic treatment-induced gingival enlargement. Also bracket placement influences the accumulation of plaque and the colonization of important periodontopathic and superinfecting bacteria, resulting in more inflammation and bleeding.[19]

The results of the study demonstrated that bacterial count decreased significantly between T0-T1 and T1-T2 in Group B and Group C. At T1 phase both the mouthwashes showed similar results. But at T2 phase Chlorhexidine was significantly better than Hiora mouthwash. This was in agreement with the study done by Lundstrom F et al that after insertion of fixed orthodontic appliances, the levels species were found to have increased, but Chlorhexidine treatments significantly reduced the number of S. mutans during the orthodontic treatment period. Beyth N et al too mentioned that sustained- release chlorhexidine varnish decreases S mutans levels in orthodontic patients with fixed appliances.[10] Yadav S et al also suggested that the antibiofilm formation action of Terminalia bellerica plant extract against Streptococcus mutans and streptococcus sobrinus was found to be a strong

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inhibitor of Streptococcus mutans.[20] The recent study also demonstrated that Chlorhexidine reduced the bacterial count more than Hiora mouthwash. This is in accordance with Saima S et al, who demonstrated that the use of chlorhexidine rinse and herbal extract mouthwash along with mechanical methods both reduced the GI in patients, but this reduction in GI was more considerable in chlorhexidine than the herbal mouthwash group.[9]

Summary and Conclusion

Mouthwashes provide important role in orthodontic treatment. Ideally, it is required that any antimicrobial or antiseptic agent used should be able to modify the oral environment by being specifically effective against pathogens without altering the normal flora. There are several types of mouthwash available in the market today worldwide. Chlorhexidine gluconate is considered the most effective antiplaque mouth rinse Herbal mouthwashes are gaining popularity capable of maintaining periodontal health, reducing dental plaque and decreasing bleeding upon brushing.

The aim of this study was to determine and compare the antibacterial efficacy of Hiora mouthwash with 0.2% chlorhexidine gluconate in orthodontic patients.

This in-vivo study was conducted in Department of Orthodontics and Dentofacial Orthopaedics, Surendera Dental College and Research Institute with 60 patients of age group between 12-30 years with fixed orthodontic appliances. Two different types of mouthwashes were used. Distilled water was used for Control group (Group A). There were two more groups Chlorhexidine group (Group B) and Hiora Group (Group C). The patients were allocated to three main groups by using Table of Randomization Method at different time points base line 1st day, 30th day and 90th day.

Statistical data was conducted on data using paired t-test, t-test, chi square test followed by Post Hoc Tukey test (HSD).The results were tabulated and graphical representations were generated. The analysis was performed using the Statistical package for Social Sciences (SPSS) (Version 24). Based on the results obtained and limitations of the study, the following conclusions were drawn:

- Microbial count increases after starting of orthodontic treatment.
- 0.2% Chlorhexidine mouthwash reduced the microbial count of Streptococcus mutans, Streptococcus sanguinis and Staphylococcus aureus after 30 days and 90 days of bonding.
- Hiora mouthwash reduced the microbial count of Streptococcus mutans, Streptococcus sanguinis and Staphylococcus aureus after 30 days and 90 days of bonding.
- 0.2% chlorhexidine gluconate mouthwash reduced gingival inflammation after 30 days and 90 days of bonding.
- Hiora mouthwash reduced gingival inflammation after 30 days and 90 days of bonding.
- Comparatively 0.2% Chlorhexidine mouthwash was better than Hiora in reduction of microbial count as well as plaque and gingivitis. For long term use of 0.2% Chlorhexidine mouthwash causes stain and impairment of taste as side effect. Hiora mouthwash can be use as an alternative to 0.2% Chlorhexidine mouthwash.

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