Original Article

Antimicrobial Effect of Mouthwashes in Patients Undergoing Orthodontic Treatment

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ABSTRACT

Objective: The purpose of the study was to determine the efficacy of three different mouthwashes in patients undergoing fixed orthodontic treatment for prevention of white spot lesions **Methods***:* The study was conducted on 30 patients undergoing fixed orthodontic treatment between the ages of 15-25years. Patients were divided into 3groups - Group 1 control group, Group 2 using freshclor for 30 sec twice daily and group 3 using HiOra mouth wash for 30sec twice daily. Samples from tooth surfaces were collected at 1st day, 30th day and 90th day interval and were incubated for 48 hours. Colonies were counted using digital colony counter. **Results***:* Freshclor and HiOra mouthwashes showed the maximum potential for the control of pathogenic organisms, and prevention of gingivitis and bacterial plaque inhibition than patients those were not using mouthwash.

Key words- Microbial activity, digital colony counter machine, mouth wash

Malocclusion is one of the most common dental disorders and is capable of increasing the risk of periodontal disease and dental caries. Optimum health of the periodontium should be maintained during the orthodontic treatment [1]. Maintaining oral hygiene is not a major problem while using removable appliances because they can be withdrawn from the oral cavity. However, fixed orthodontic appliances provide an additional surface for the collection of food debris and plaque resulting in increased bacterial colonization and subsequently, accentuate their luxuriant growth in supragingival and sub gingival region. The development of white spot lesions is almost inevitable when oral hygiene is poor. Demineralization is more commonly seen on the buccal surfaces of orthodontically treated teeth than on untreated teeth and it leads to increase in the incidence of caries [2]. Several methods are required to reduce the microbial growth and plaque formation, mechanical or chemical method. In chemical method, antimicrobial mouth rinses are recommended as an adjunct to mechanical plaque control. Freshclor and hiora mouthwash has been used in treating halitosis, plaque, and gingivitis [3-5]. Hence, this study was designed to find out the most effective mouthwash in patients undergoing fixed orthodontic treatment so that white spot lesions can be prevented.

MATERIALS AND METHODS

The current study was conducted as a randomized controlled trial in the Department of Orthodontics and Dentofacial Orthopaedics, Chhattisgarh Dental College and Research Institute Rajnandgaon. The study sample included 30 patients in the age group between 15 to 25 years. Patients requiring fixed orthodontic treatment and devoid of any clinical evidence of pulpitis and impacted teeth were included. Exclusion criteria was presence of more than 5mm crowding of teeth, presence of periodontal

pathology, those taking any antibiotics, anti-inflammatory drugs and patients having systemic diseases. 30 patients were randomly divided into three groups and were kept under a specific oral hygiene regime.

1. Group 1 (Control group):- 10 patients undergoing regular orthodontic care (brushing the teeth with modified bass technique twice a day).
2. Group 2:- 10 patients undergoing regular orthodontic care and prescribed with 10 ml Freshclor mouthwash for 30 seconds twice daily.
3. Group 3:- 10 patients undergoing regular orthodontic care and prescribed with 15 ml Hiora mouthwash for 30 seconds twice daily.

Fig 1 - Plaque Collection, Before And After

Samples were taken on 1st day, 30th day and 90th day interval. Microbiological trials were carried out on buccal surface of upper and lower first molars of all the patients at all intervals. Patients were examined and their dental plaque was scored by Silness and Loe "Plaque Index", and their gingival status was scored by Loe and Silness "Gingival Index". Patients were demonstrated with modified bass brushing technique, using super soft tooth brush and use of mouthwash. Oral prophylaxis was done in all the cases, to make tooth surface free from calculus, plaque and other deposits. Patients were instructed to follow their group regimes. After each interval, swab sample were collected and sent to the laboratory within 30 minutes where it was spread on small petridishes containing nutrient agar culture media for colonization.

Petridishes were incubated at 37°C in micro-aerophilic environment at 5% CO_2 for 48 hours. The colonies were counted using a digital colony counter machine and microbial counts were expressed as colony forming unit (CFUs/ml).

Statistical Analysis: Statistical analysis was performed Paired sample t-test was used to analyze the difference between all the study groups.

RESULTS

Comparative analysis of percentage reduction of microbial counts between the study groups is depicted in table 1 indicating the highest reduction in group 2 i.e. Freshclor group (55.44-66.01%). The mean reduction in the bacterial count (table 2) was highest in group 2 (60.068±3.310), and the lowest in group 1 (8.695±1.278), while intermediate in the group 3 (47.473±1.052) with all 3 values being highly significant (p=0.000). Table 2 shows the comparison between the three study groups for microbial count, where group 1 shows the maximum number of microbial count (51.373±3.967).

Table 1- Mean percentage reduction of microbial count

Parameters		Group 1	Group 2	Group 3
Mean ± SD		8.69±1.27	60.07±3.31	47.47±1.05
Std. Error Mean		0.404	1.047	0.333
t-test		21.51	57.38	142.66
Df		9	9	9
p-value		0.000*	0.000*	0.000*
95% CI	Upper	9.61	62.43	48.23
	Lower	7.78	57.700	46.720

Table 2 - Intergroup comparison of percentage reduction of microbial count for all the study groups

Intergroup comparison	Mean ± SD	Standard Error of Mean	t-test	N	p-value	95% CI	
						Lower	Upper
Group 1 vs. 2	-51.37±4.02	1.254	-40.95	9	0.000*	-54.21065	-48.53535
Group 1 vs. 3	-38.78±1.41	0.449	-86.42	9	0.000*	-39.79304	-37.76296
Group 2 vs. 3	12.59±4.05	1.053	11.96	9	0.000*	10.21207	14.97793

Table 3 analyses the significance relation between the study groups to evaluate the change in plaque index after 3 months. It was found that group 1 (1.55±1.10) showed a non-significant relation; whereas group 2 (12.05±8.52) and group 3 (10.29±7.28) showed a significant relation. The intergroup comparison between plaque index showed the least difference in reduction of Plaque Index between Group 2 and Group 3 (9.19±0.98), and the maximum difference between Group 1 and Group 2 (49.17±0.66) followed by Group 1 and Group 3 (39.98±0.56).

Table 3 - Change in the plaque index after 3 months

Groups	Mean±SD (n=10)	t-test	p-value
Group 1	1.56±1.10	21.260	0.073** NS
Group 2	12.05±8.51	52.619	0.022* S
Group 3	10.29±7.32	38.255	0.039* S

Table 4 shows one sample t-test to analyze the change in gingival index after 3 months. We found that group 1 (1.36±0.96) showed a non-significant relation; whereas groups 2 (10.28±7.27) and 3 (7.73±5.46) showed a significant relation. The intergroup comparison of plaque index is shown in table 5 and was found to be highly significant. The least difference in reduction of gingival index was seen between group 2 and 3 (7.93±2.48), and the maximum difference was seen between group 1 and 2 (49.09±2.63) followed by group 1 and 3 (41.16±0.1.69).

Table 5 - Intergroup comparison of Plaque Index

DISCUSSION

The idea of employing a chemical agent which would act in an identical manner to a toothbrush and remove bacteria from the tooth surface is an attractive proposition. This chemical agent contained in mouth rinse would be expected to reach all tooth surfaces and thereby be totally effective. Chemical plaque removal agents have attracted the terminology of "the chemical toothbrush" [6]. In this study, two different mouthwashes were used - Freshclor mouthwash and Hiora mouthwash.

Table 4 - Change in gingival index after 3 months

Groups	Mean±SD (n=10)	t-test	p-value
Group 1	1.36±1.01	14.176	0.076** NS
Group 2	10.28±7.27	42.546	0.028* S
Group 3	7.73±5.46	35.127	0.037* S

The Freshclor mouthwash contains a stabilized chlorine dioxide. Chlorine dioxide (ClO_2) is an oxidizing agent with known bactericidal, virucidal and fungicidal properties. It inhibits microorganism growth by disruption of the transport of nutrients across the cell membrane. ClO_2 oxidatively consumes and inactivates salivary biomolecules, including pyruvate, methionine, trimethyl-arnine, tyrosine and glycine; thereby, exerting its antimicrobial effect.

Intergroup comparison	Mean ± SD	Standard Error of Mean	t-test	N	p-value	95% CI	
						Lower	Upper
Group 1 vs 2	49.09±2.63	0.83262	-58.956	9	0.000*	-50.97152	-47.20448
Group 1 vs 3	41.16±2.01	0.53382	-77.095	9	0.000*	-42.36259	-39.94741
Group 2 vs 3	7.93±3.02	0.78580	10.095	9	0.000*	6.15540	9.71060

A reduction product of chlorine dioxide, chlorite also acts as a reactive oxidant towards biomolecules like endogenous thiols such as cysteine. ClO_2 being highly soluble in water can penetrate into the biofilm rapidly and exert its antimicrobial action. Stabilized ClO_2 refers to the generation and subsequent sequestration of chlorite, which allows for its storage and increases its shelf-life [6-7]. Hiora mouthwash has antimicrobial, antiplaque, antiseptic, analgesic and refreshing properties. It is an herbal preparation, made from a combination of natural herbs with beneficial properties of-

1. Anticariogenic and antiplaque. –Pilu (*Salvadorapersica)*.
2. antibacterial, anti-inflammatory, and immunity booster - Bibhitaka (*Terminalia bellerica*)
3. antioxidant, antimicrobial, and plaque inhibiting properties - Nagavalli (*Piper betle*)
4. Essential oils of Gandhapurataila (Gaultheria fragrantissima) possess antimicrobial, anti-inflammatory, and analgesic properties.
5. Oil extracted from Ela (*Elettaria cardamomum*) is a potent antiseptic that is known to kill bacteria producing bad breath.
6. Peppermintsatva (*Mentha*spp) Acts as a natural mouthfreshener.
7. Yavanisatva (Trachyspermumammi) also has antimicrobial properties [8].

Fard BK et al (2011) evaluated the effect of Orthokin, Listerine and Oral B mouthwash on the Mutans Streptococcus (MS) and plaque index in orthodontic patients. These factors were measured before and after 21 days of mouthwash use. They concluded that Ortho-kin was more effective than the other mouthwash in reducing MS and plaque index around the orthodontic brackets. In the present study, modified bass brushing technique was advised for the cleaning of all the surfaces of tooth. It is standard brushing technique and widely accepted by the dental professionals. Therefore; recommended modified bass techniques were used by all participants and were advised to use same type of tooth brush and toothpaste [3]. The data thus collected were subjected to statistical analysis by classifying into three categories: 1) Total colony forming units (CFU); 2) plaque accumulation; and 3) gingival condition.

Total Colony Forming Unit: A comparison of the total CFU of various mouth washes revealed that Freshclor mouthwash was the most effective in reducing total CFU followed by Hiora mouthwash than the control group.

Plaque accumulation: Freshclor mouthwash was most efficient in reducing plaque from the tooth surface, on which edgewise appliance is fixed (12.05±8.52), followed by Hiora mouthwash (10.29±7.28), and least efficient was the routine care (1.56±1.10). This was in accordance with the study conducted by Sarangal V et al (2014) who also found significant reduction in plaque and gingival indices after use of both Hiora and Chlorhexidine mouthwash. Kaur R et al (2014) also demonstrated highly significant reduction in gingival index and OHI-S after use of Hiora mouthwash. Abhishek K et al (2013) and Kumar S et al (2016) also showed a significant reduction in Plaque and Gingival Index after use of ClO_2 mouthwash which was in accordance with our study [10,11]. However, none of these studies were carried out in orthodontic patients.

Gingival condition: Freshclor mouthwash was very effective in reducing gingivitis (10.28±7.27) followed by Hiora mouthwash (7.73±5.46). The routine care was the least effective (1.36±0.96) method in reducing gingivitis. A similar comparative assessment of plaque index in different groups again reveals highly significant differences between different groups (control vs. Freshclor; control vs. Hiora and Freshclor vs Hiora mouthwash)**.** The results thus obtained may be due to variation in composition of mouth washes. The reduction in total CFU, plaque accumulation, gingivitis from the base line may be attributed to proper brushing method and conscious oral rinsing. The study showed that the use of mouthwashes in conjunction with oral hygiene procedures during longer orthodontic treatment plays an important role in maintaining the oral hygiene of the patients.

Patients derive both functional and aesthetic benefits from orthodontic treatment. Based on the findings of this study, it is evident that patients wearing orthodontic appliances have a problem in maintaining good oral hygiene. Inadequate oral home care among orthodontic patients may make them more prone to develop gingivitis during orthodontic treatment. Therefore, educating and motivating these patients, to maintain their oral health and providing recommendations for oral home care aid to improve their compliance, remains the cornerstone for achieving optimal oral hygiene results. It is, therefore, essential that oral hygiene instructions and a hygiene maintenance program not be overlooked during orthodontic treatment.

CONCLUSION

Freshclor mouthwash showed the maximum potential for the control of pathogenic organisms, and prevention of gingivitis and bacterial plaque inhibition. Hiora used as mouthwash is found to be effective in reduction of total colony forming units, gingivitis and plaque accumulation but is less effective than Freshclor. Patients who were not using mouthwash were found to be least effective.

REFERENCES

1. Aghili H, Jafari A, Nadoushan, Herandi V. Antimicrobial Effect of *Zataria Multiflora* Extract in Comparison with Chlorhexidine Mouthwash on Experimentally Contaminated Orthodontic Elastomeric Ligatures. J Dent (Tehran). 2015; 12(1): 1–10

2. Isotupa KP, Gunn S, Chen CY, Lopatin D, Makinen KK. Effect of polyol gums on dental plaque in orthodontic patients. Am J Orthod Dentofac Orthop. 1995; 107: 497-504.

3. Kumar R, Kapoor D, Jain A, Tandon P. Microbicidal Effect of Different Mouthwashes on Patients Undergoing Fixed Orthodontic Treatmnent. J Ind Orthod Soc. 2006; 39:139-146.

4. Dehghani M, Abtahi M, Sadeghian H, Shafaee H, Tanbakuchi B. Combined chlorhexidine-sodiumfluoride mouthrinse for orthodontic patients: Clinical and microbiological study. J Clin Exp Dent. 2014. 7(5): e569–e575.

5. Kumar SY, Acharya S, Urala A, Pentapatid K. Effect of *Aloe vera*, chlorine dioxide, and chlorhexidine mouth rinses on plaque and gingivitis: A randomized controlled trial. J Oral Biol Craniofac Res. 2016; 6(1): 54-8

6. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontal 1988; 15: 488-498.

7. Yadav S, Kini V, Padhye A. Inhibition of Tongue Coat and Dental Plaque Formation by Stabilized Chlorine Dioxide Vs Chlorhexidine Mouthrinse: A Randomized, Triple Blinded Study. J Clin Diagn Res. 2015; 9(9): ZC69–ZC74

8. Bagchi S, Saha S, Jagannath GV, Reddy VK, Sinha P. Evaluation of efficacy of a commercially available herbal mouthwash on dental plaque and gingivitis: A double-blinded parallel randomized controlled trial. J Indian Assoc Public Health Dent 2015; 13: 222-7.

9. Fard BK, Ghasemi M, Rastgariyan H, Sajjadi SH, Emami H, Amani M, et al. Effectiveness of Mouth Washes on Streptococci in Plaque around Orthodontic Appliances. ISRN Dent. 2011; 954053

10. Sarangal V, Kaur S ,Singh S, Suman S, Kaur N, Malhotra S. Evaluation of clinical efficacy and safety of commercially available herbal mouthwash (hiora) in comparison with chlorhexidine mouthwash (aster-x) in improving oral health in patients undergoing dental procedures. BFUDJ, 2014; 5(2): 38-42.

11. Kandwal A, Ghani B. A comprative evaluation of effect ofchlorine dioxide mouthrinse on plaque induced gingivitis and oral malodor: a clinical study. Int J Dent Health Sci, 2014; 1: 24-33.

12. Shetty S, Pillai S, Rahul A. Asian Journal of Pharmaceutical Technology & Innovation, 2013; 1(3):1-8.