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RESEARCH ARTICLE

TO EVALUATE THE EFFECT OF DIFFERENT TYPES OF MOUTH WASHES ON THE LEVEL OF STREPTOCOCCUS MUTANS IN THE INITIAL PHASE OF ORTHODONTIC TREATMENT

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ABSTRACT

Introduction: Dental malocclusions are one of the most common oral health problems and are associated with difficulties in maintaining oral hygiene. Mouthwashes may affect the plaque formation and reduces the number of streptococcus mutan count. **Aims and objectives:** To evaluate the effect of alcoholic, non-alcoholic and organic mouthwash on the level of streptococcus mutans in the during the early phase of orthodontic treatment. **Material and Methods**: 120 patients aged between 12 -20 years who visited the department were selected and randomly allocated into four l groups. Microbial records and periodontal parameters obtained before bonding (T_0), 1 week (T_1), 2 week (T_2), 3 week (T_3), and 4 week (T_4) after bonding and colonies were counted under Stereomicroscope and the results were expressed as colony forming units per milliliter and analysis was done using the SPSS ($p<0.05$). **Conclusion**: On comparison it was found that both organic mouthwash (Hiora) and non alcoholic mouthwash (Rexidin Plus) showed comparable clinical and microbiological results.

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INTRODUCTION

Dental malocclusions are one of the most common oral health problems and are associated with a number of complications. Most orthodontic patients have difficulties in maintaining oral hygiene (Shankar, 2014). Fixed orthodontic appliances with the presence of brackets and ligatures create new retention areas for microbial dental plaque that is reported most important etiological factor in periodontal inflammation and decalcification of enamel (Uzumer, 2014). The relationship between orthodontic procedures and periodontal status is considered a challenge, especially periodontal health during and after orthodontic treatment. Orthodontic treatment is a double-action procedure regarding the periodontal tissues, which may be sometimes very significant in increasing the periodontal health status and sometimes a harmful procedure which can be followed by several types of periodontal complications such as "gingival recessions, bone dehiscence, gingival invaginations and formation of gingival pockets (Antony, 2013)".

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The white spot lesions are considered to be the precursor of enamel caries and, has been attributed to prolonged accumulation and retention of bacterial plaque on the enamel surface adjacent to the orthodontic appliances (O'Reilly, 1997). Roughness of the composite surface predisposes to rapid attachment and growth of oral microorganisms. Clinical observation is at the periphery and gingival to bracket bases at the junction between bonding resin and the enamel, just peripheral and commonly gingival to the bracket base (Sukontapatipark, 2001). Other favoured sites for such accumulation are around the cervical margins of the teeth, under the bands in areas where the cementing medium has washed out, on the resin surfaces adjacent to bonding attachments. Despite recent advances in orthodontic material, there has been no decrease in the prevalence of enamel demineralization near the bracket-tooth junction (Shankar, 2014). Patients who undergo fixed mechanotherapy which led to increased proportions and absolute numbers of salivary *Streptococcus mutans* (Rosenbloom, 1991). The initial affinity of bacteria to solid surfaces is mostly due to electrostatic and hydrophobic interactions. The composition as well as rate of saliva secretion may also effect bacterial adherence. Among other species, *Streptococcus mutans* (SM) and Lactobacillus (LB) bacteria, have been identified with high concentration in

oral flora and have higher adhesion capacity on brackets, predisposing to dental caries (Uzumer, 2014). Streptococcus mutans and Streptococcus sobrinus are the most important bacteria in the pathogenesis of dental caries due to many epidemiological, experimental and animal studies. This is due to their ability of rapid lactic acid formation from dietary carbohydrates, mainly sucrose and glucose (Al-Mudallal *et al*., 2008). Consequently, removal or modification of bacterial plaque is the principal goal in preventing inflammatory periodontal diseases. Presently mechanical and chemical methods of plaque alteration are the two main approaches available (Fine, 1985). Adequate plaque removal through mechanical methods alone may be difficult in patients undergoing fixed orthodontic treatment (Kumar, 2006). Hence chemotherapeutic agents may have a key role as adjuncts to mechanical methods for preventing and treating periodontal diseases (Santos, 2003). These chemical antimicrobial substances are capable of inhibiting bacterial adhesion, colonization and metabolic activity ultimately affecting the bacterial growth (Kulkarni, 2003). The antiplaque and ant gingivitis effectiveness of mouthrinses containing antimicrobial agents has been well documented. In particular, the effectiveness and safety of a mouthrinse containing a fixed combination of essential oils has been demonstrated in a number of long term clinical trials, both with respect to reduction of existing supragingival plaque and gingivitis and inhibition of newly forming plaque and gingivitis (Fine *et al*., 2007). Several bisguanide antiseptics possess anti-plaque activity, including chlorhexidine, alexidine and octenididne. Chlorhexidine is effective against both gram positive and gram negative bacteria. Its antibacterial action is due to an increase in cellular membrane permeability followed by coagulation of cytoplasmic macromolecules (Eley, 1999). Listerine antiseptic is a topically active mouthwash which has been shown to significantly reduce preformed dental plaque and to inhibit the development of plaque following prophylaxis (Fine, 1985). Recently there has been considerable interest in the non-ionic antimicrobial, Triclosan. Mouth rinsing containing tricloan have been shown effective plaque inhibitors both alone and combined with tooth brushing (Moran, 1994). Many studies have been done to evaluate the effect of chlorhexidine mouthwash and listerine mouthwash during orthodontic appliance placement. Natural products have been used for medicine purposes throughout the world for thousands of years. Many of them have pharmacological properties (antimicrobial properties and anti-inflammatory properties). Medicated oral rinses usually contains antimicrobial agents, such as chlorhexidine gluconate which is very potent chemoprophylactic agent, it has a broad spectrum action especially against Mutans Streptococci group .Thus organic mouthwashes may affect the plaque formation and reduces the number of streptococcus mutan count. Since you hardly find a study in literature which evaluate the effect of alcoholic, non-alcoholic and organic mouth wash in reducing streptococcus mutans count during orthodontic appliance placement. So the objective of the present study was to evaluate the effect of alcoholic, non-alcoholic and organic mouthwash on the level of streptococcus mutans in the during the early phase of orthodontic treatment.

MATERIALS AND METHODS

The present study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics and Department of Microbiology Sample selection was done on the basis of the following inclusion criteria:

- Patient aged between 12-20 years of age.
- Good general health.
- No sign of destructive periodontal disease and no more than 4 mm gingival pocket depth and free of dental plaque.
- No regular medication with anti-inflammatory compounds and no history of allergy.
- Mild to moderate crowding of teeth.
- No usage of antibiotics in the 3 months before beginning of study.
- Patient should be non-smokers.

120 patients aged between 12 -20 years who visited the department were selected and randomly allocated for groups.

Group I was taken as a control group before any orthodontic appliance placement (To). This control group was further divided into 3 groups of 40 each based on the type of mouth wash prescribed.

Group II: This group consists of 40 patients and was directed to use alcohol containing mouth wash (Listerine by Johnson and Johnson) twice a day for 30 seconds after orthodontic appliance placement.

Group III: This group consists of 40 patients and was directed to use non-alcohol containing mouth wash (Rexidine by Abbott Healthcare Pvt, Ltd.) twice a day for 30 seconds after orthodontic appliance placement.

Group IV: This group consists of 40 patients and was directed to use organic mouth wash (Hiora by Himalayan Global Holdings Ltd.) twice a day for 30 seconds after orthodontic appliance placement. All the patients were given oral hygiene instructions with proper brushing technique (Bass method) and asked to brush twice a day before the start of treatment. Patients were then instructed to use 10 ml of mouth wash for 1 minute twice a day after brushing and they are informed not to eat or drink anything for 1 hour after using mouth wash. Informed consent forms were signed by all subjects with written descriptions of the study design. The study was approved by the regional Ethical Committee.

Sample Collection: At base line, before orthodontic treatment, the upper right central incisor, upper right first premolar, lower left central incisor, and lower left first premolar teeth were isolated with cotton rolls and the area was allowed to dry. Biofilm from the above mentioned teeth of all the patients was picked gently (figure1) with the help of a microbrush and the samples were transferred to a tube containing nutrient broth (figure2)containing peptic digest of animal tissue, sodium chloride, beef extract and yeast extract. Various clinical parameters were recorded including Gingival index as suggested by Loe (1967), Plaque index as described by Loe, (1967) Gingival bleeding index as described by Ainamo J and Bay (1975) and Pocket depth measured with the help of Williams periodontal probe. After the clinical examination, all the patient received fixed orthodontic appliances that consisted of orthodontic bracket on the incisors, canine, premolars and orthodontic bands on first molars. Pre adjusted edgewise metal brackets were used in all the patients with 0.014 nitinol arch wires for initial leveling.

Microbial records and various periodontal parameters were obtained before bonding (T_0), (Shankar, 2014)

week (T_1), 2 week (T_2), 3 week (T_3), and 4 week (T_4) after bonding.

Isolation of bacteria from dental plaque: The plaque samples were inoculated in nutrient broth aseptically followed by incubation at 37º C for 12 - 24 hours. After incubation, nutrient broth got turbid that indicates growth of bacteria. Serial 10^{-4} dilution from turbid nutrient broth was prepared and 1 ml sample was inoculated on Tryptone Yeast Extract Cystine with sucrose without bacitracin agar base (TYCSB) plates (recommended for selective isolation of streptococcus mutans) following incubation for 24 - 48 hours at 37^{o} C. Microbial samples taken were cultivated (figure3) and analyzed by the same examiner in the department of microbiology.

Counting of colony forming unit (cfu): By using serial dilution technique, 10 fold dilution was prepared to know the dilution factor. and colony forming units (CFU) were calculated by using the following formula:

Then the number of colonies were counted under Stereomicroscope (figure 4) and the results were expressed as colony forming units per milliliter. The SPSS (Statistical Package for Socia Sciences) software package was used for statistical calculation and ANOVA test was used for group comparison.

RESULTS

The present study was designed to compare the different types of mouthwashes at different intervals of time, to evaluate the level of streptococcus mutans count in early phase of orthodontic treatment. The statistical analysis was done using the SPSS (statistical package for social sciences) software. The significance in the difference in the means of various parameters was done by using analysis of variance (ANOVA) and Tukey's Post Hoc Test for multiple comparisons as shown in Table I , II and III and Graph 1,2 and 3. Table IV and Graph 4. Showed The Mean Value Of Streptococcus Mutan(10^4 CFU/ml) at Different Intervals Of Time In Alcohol Containing (Listerine), Non Alcohol Containing mouthwash (Rexidin Plus) and Organic Mouthwash Group (Hiora). Table v: Comparison of means of S mutans in three groups at different intervals by one way ANOVA Table vi Multiple Comparisons of means of streptococcus mutans in three types of mouth wash groups by Post Hoc Tukey HSD Tests.

DISCUSSION

Dental caries, gingival disease, and periodontal disease are the most frequently occurring oral diseases in the world. The use of tooth brushing and fluoride toothpaste appeared to be almost universal. There is difficulty in effectively removing plaque by only using tooth brushing and flossing, as these measures do not remove plaque completely. During fixed orthodontic therapy, braces, bands, wires, and other attachments make it difficult for the patient to perform mechanical oral hygiene procedures; this difficulty results in plaque accumulation, which is the main cause of demineralization. Failure to maintain proper oral hygiene leads to tooth damage, consequently affecting the orthodontic treatment. Therefore, the levels of cariogenic pathogens should be constantly reduced during orthodontic treatment. Better oral hygiene can be achieved if a sustained delivery of an anticariogenic drug is used, because it maintains therapeutic levels of the drug in the oral cavity. The incorporation of broad spectrum antimicrobial mouthrinses as adjuncts to patient's daily oral hygiene regimens has assumed greater importance with the recognition that most individuals are unable to consistently maintain adequate levels of plaque control using mechanical methods alone. There is a necessity for adjuncts, in addition to mechanical aids for maximum plaque control. A variety of subsequent studies have demonstrated the antiplaque and anti-gingivitis effectiveness of chlorhexidine mouthrinses as adjuncts to usual oral hygiene methods. As a result, 0.2% chlorhexidine mouthrinses became widely used in many European countries. Till present, chlorhexidine mouthwash is considered as gold standard for the best plaque control agent (Shankar *et al*., 2014). However, these rinses have side effects, such as enamel discoloration, mucosal erosion, taste disturbance, mouth burning, dry mouth, carcinogenic effects and the smoothing of composite materials, which limits their usage.

Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeias as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. Natural herbs like neem, tulsi, triphala, clove oil, ajwain, etc. have been used alone or in combination and have been scientifically proven to be safe and effective medicine against various oral health problems like bleeding gums, halitosis, mouth ulcers and decay. The major strength of these natural herbs is that their use has not been reported with any side effect till date. One such herbal product is Hiora, a herbal mouthwash known for its antiseptic, antimicrobial, antiplaque and analgesic property. Since various studies have been done to evaluate the effect of chlorhexidine mouthwash and listerine mouth wash on the level of streptococcus mutans count, but no study have been done to evaluate the effect of organic mouth wash on the level of streptococcus mutans count. So the objective of the study was to evaluate the effect of different types of mouth washes on the level of streptococcus mutans in the initial phases of orthodontic treatment. In the present study, the mean value of gingival index, periodontal index, gingival bleeding index and pocket depth are shown in (Table I and Graph 1**)**. When alcohol containing mouth wash (Listerine) was instructed to use after orthodontic appliance placement**,** it was observed that with the progression of time, there was slight decrease in the mean value of gingival index. The mean value was highest at To and lowest at T4. The mean value of streptococcus mutans (10^4 CFU/ml) in alcoholic mouthwash was found to be highest at T0 and was found to be least at T4 as shown in Table IV and Graph 4. This might be due to the fact that the incorporation of alcohol act as a solvent for other active ingredients; it has antiseptic properties and acts as a preservative. Alcohol on its own does cause damage to the oral mucosa and includes epithelial atrophy, decease in basal cell size atrophy with associated hyper regeneration. The prime metabolite of alcohol is acetaldehyde and the bulk of the metabolism of alcohol is carried out in the liver, there is evidence that alcohol metabolism could occur in the oral cavity and that various bacteria in plaque can metabolize alcohol to

Table 1. Showed longitudinal changes in periodontal measurements in alcohol containing mouthwash group (Listerine)

	T0	T1	T2	T3	T4
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Gingival index	1.78 ± .26	1.76 ± .26	1.70 ± .30	1.60 ± .26	1.49 ± .21
Plaque index	.48 ± .31	.42 ± .27	.40 ± .25	.36 ± .26	.34 ± .24
Gingival bleeding index	.23 ± .07	.21 ± .4	.15 ± .07	.11 ± .07	.08 ± .06
Pocket depth	2 ± .36	1.96 ± .36	1.93 ± .36	1.88 ± .36	1.85 ± .36

Table 2. Showed longitudinal changes in periodontal measurements in non alcohol containing mouthwash group (Rexidin Plus)

	T0	T1	T2	T3	T4
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ±S.D	Mean ±S.D
Gingival index	1.78 ± .28	1.26 ± .25	.59 ± .42	.21 ± .18	.12 ± .12
Plaque index	.56 ± .34	.37 ± .40	.29 ± .40	.21 ± .40	.11 ± .40
Gingival bleeding index	.23 ± .07	.19 ± .07	.16 ± .07	.12 ± .07	.05 ± .07
Pocket depth	2.04 ± .35	1.9 ± .35	1.1 ± .35	.78 ± .12	.36 ± .35

Table 3. Showed longitudinal changes in periodontal measurements in organic mouthwash group (Hiora)

	T0	T1	T2	T3	T4
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Gingival index	1.75 ± .27	1.53 ± .32	1.36 ± .29	1.10 ± .43	.83 ± .55
Plaque index	.56 ± .36	.32± .36	.11 ± .36	.09 ± .37	.02 ± .36
Gingival bleeding index	.22 ± .07	.15 ± .07	.11 ± .05	.07 ± .04	.03 ± .03
Pocket depth	1.9 ± .33	1.8 ± .33	1.79 ± .29	1.65 ± .12	1.57 ± .34

Table 4. Showed The Mean Value Of Streptococcus Mutan (10^4 CFU/ml*) at Different Intervals Of Time In Alcohol Containing (Listerine), Non Alcohol Containing mouthwash (Rexidin Plus) and Organic Mouthwash Group (Hiora)

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
T	Organic	9.84	4.239	8.09	11.59	3	19
0	Alcoholic	7.84	2.461	6.82	8.86	0	12
	Non-Alcoholic	9.68	2.911	8.48	10.88	5	16
T	Organic	11.16	5.383	8.94	13.38	5	22
1	Alcoholic	9.08	4.564	7.20	10.96	5	19
	Non-Alcoholic	10.88	4.076	9.20	12.56	6	19
T	Organic	5.28	1.990	4.46	6.10	1	9
2	Alcoholic	6.92	1.847	6.16	7.68	4	11
	Non-Alcoholic	6.36	1.912	5.57	7.15	3	10
T	Organic	4.76	1.985	3.94	5.58	1	9
3	Alcoholic	6.16	2.095	5.30	7.02	3	11
	Non-Alcoholic	5.80	1.780	5.07	6.53	3	8
T	Organic	3.36	1.655	2.68	4.04	1	7
4	Alcoholic	5.72	2.283	4.78	6.66	3	10
	Non-Alcoholic	3.24	1.640	2.56	3.92	0	6

*--Colony Forming Unit Per Milliliter

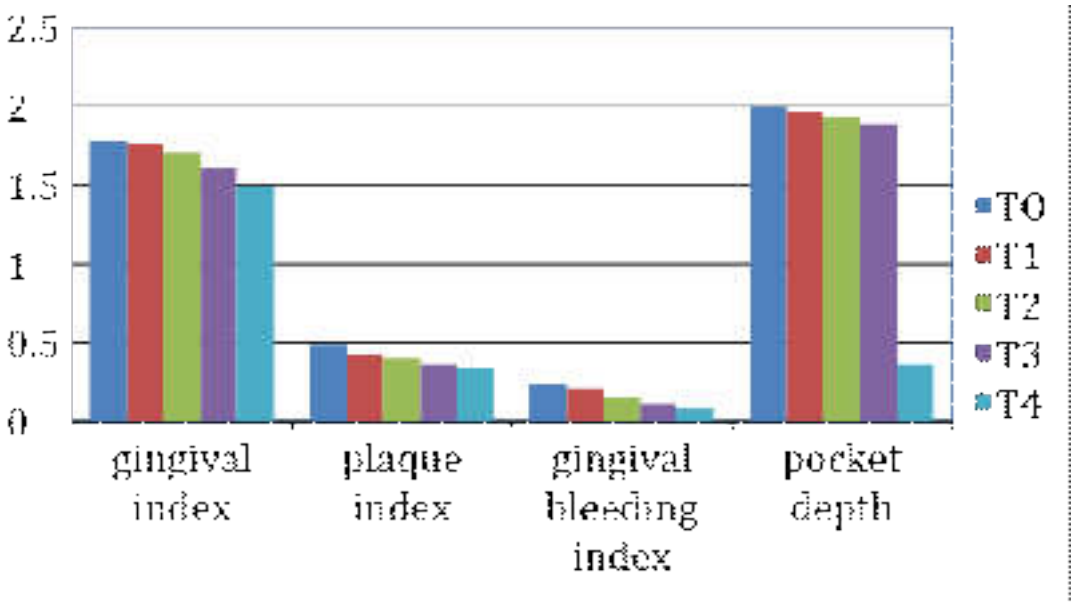
Table 5. Comparison of means of S mutans in three groups at different intervals by one way ANOVA

		Sum of Squares	df	Mean Square	F	P value
T0	Between Groups	61.760	2	30.880	2.850	.064
	Within Groups	780.160	72	10.836		
	Total	841.920	74			
T1	Between Groups	63.707	2	31.853	1.439	.244
	Within Groups	1593.840	72	22.137		
	Total	1657.547	74			
T2	Between Groups	34.747	2	17.373	4.727	.012
	Within Groups	264.640	72	3.676		
	Total	299.387	74			
T3	Between Groups	26.427	2	13.213	3.448	.037
	Within Groups	275.920	72	3.832		
	Total	302.347	74			
T4	Between Groups	97.787	2	48.893	13.786	.000
	Within Groups	255.360	72	3.547		
	Total	353.147	74			

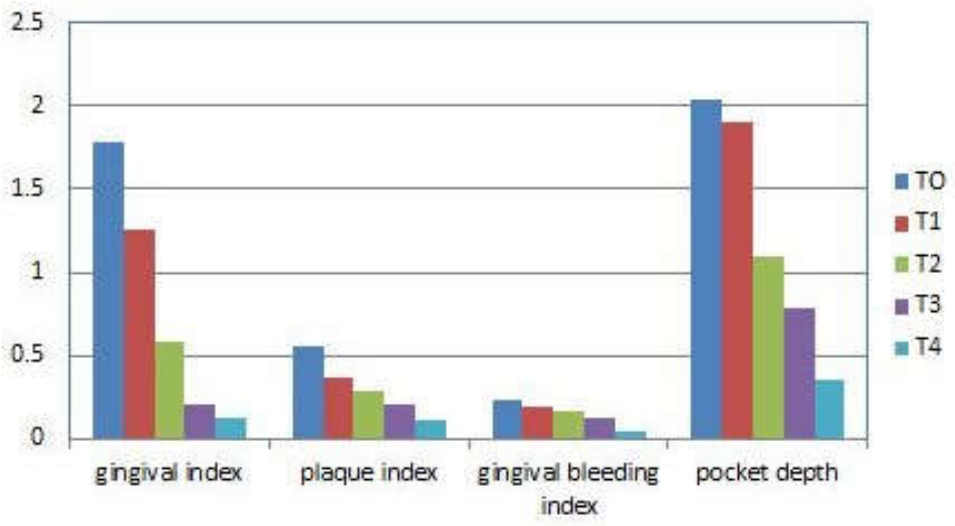
Table 6. Multiple Comparisons of means of streptococcus mutans in three types of mouth wash groups by Post Hoc Tukey HSD Tests

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
T0	Organic	Alcoholic	2.000	.931	.087	-.23	4.23
		Non-Alcoholic	.160	.931	.984	-2.07	2.39
	Alcoholic	Non-Alcoholic	-1.840	.931	.125	-4.07	.39
T1	Organic	Alcoholic	2.080	1.331	.268	-1.10	5.26
		Non-Alcoholic	.280	1.331	.976	-2.90	3.46
	Alcoholic	Non-Alcoholic	-1.800	1.331	.371	-4.98	1.38
T2	Organic	Alcoholic	-1.640*	.542	.010	-2.94	-.34
		Non-Alcoholic	-1.080	.542	.122	-2.38	.22
	Alcoholic	Non-Alcoholic	.560	.542	.559	-.74	1.86
T3	Organic	Alcoholic	-1.400*	.554	.036	-2.73	-.07
		Non-Alcoholic	-1.040	.554	.152	-2.37	.29
	Alcoholic	Non-Alcoholic	.360	.554	.003	-.97	1.69
T4	Organic	Alcoholic	-2.360*	.533	.000	-3.63	-1.09
		Non-Alcoholic	.120	.533	.972	-1.15	1.39
	Alcoholic	Non-Alcoholic	2.480*	.533	.000	1.21	3.75

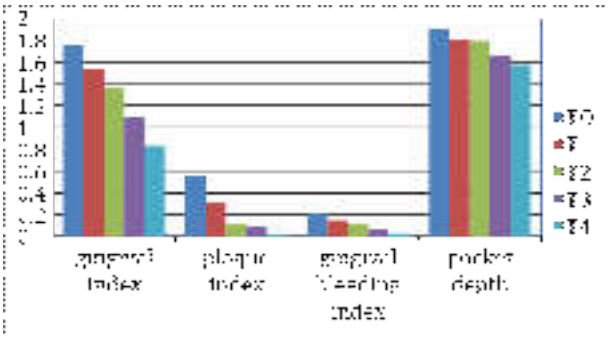
*. The mean difference is significant at the 0.05 level.



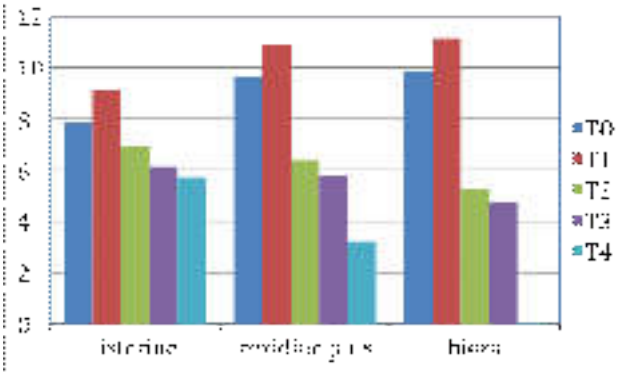
Graph 1. Showed the comparison of Longitudunal Changes of Periodontal Measurements In Alcohol Containing Mouthwash (Listerine)



Graph 2. Showed the comparison of Longitudunal Changes of Periodontal Measurements In Non Alcohol Containing Mouthwash (Rexidin Plus)



Graph 3. Showed the comparison of longitudunal changes of periodontal measurements in organic mouthwash (Hiora)



Graph 4. Showed the comparison of mean value of streptococcus mutans at different intervals of time in alcohol containing (listerine), non alcohol containing mouthwash (Rexidine Plus) and organic mouthwash group

Figure 1. Showed the collection of sample from the patient's mouth with the help of Microbrush

acetaldehyde. This may support the only account for why patients with poor oral hygiene are at an increased risk of oral cancer. In addition to the possible risk of oral cancer, alcohol containing mouthwashes are also reported to have other adverse effects on oral structures and functions. These include burning mouth, drying of the oral mucosa, softening effects on composite filling materials and mucosa pain. As clinicians, we would prefer to recommend mouth rinses that do not have these side effects. Therefore, there is a need to identify alternative, alcohol-free solutions for sensitive patients and those who do not wish to use alcohol-containing mouth rinses, such as former alcoholics or those whose religions prevent the consumption of alcohol. These results were accordance with the study conducted by Gjermo *et al*. they concluded that alcohol showed only a slight antibacterial efficacy against the oral bacteria (Gjermo, 1970). In the present study when non-alcohol containing mouth wash (Rexidin Plus)was instructed to use

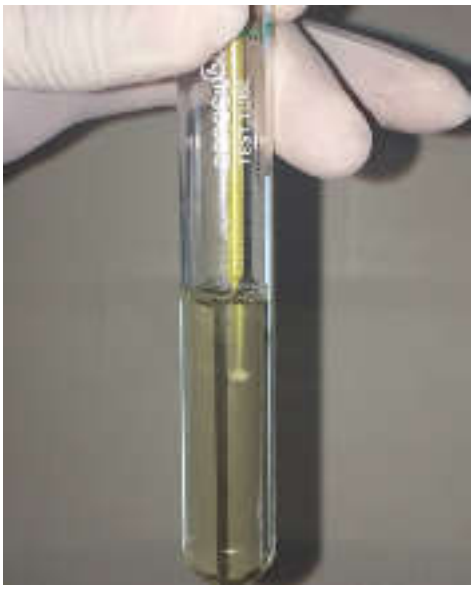


Figure 2. Showed the transfer of the sample in a test tube containing transport media (Nutrient broth)

Figure 3. Showed the inoculation of the sample on the agar plate for isolation of streptococcus mutans

Figure 4. Showed the inoculation of the sample on the agar plate for isolation of streptococcus mutans under stereomicroscope

after orthodontic appliance placement (Table 2 and Graph 2), it was observed that with the progression of time, there was moderate decrease in the mean value of gingival index , plaque index , gingival bleeding index and pocket depth. The mean value was highest at To and lowest at T4 as shown in Table II. The mean value of streptococcus mutans (10^4 CFU/ml) in alcoholic mouthwash was found to be highest at T0 and was found to be least at T4 as shown in Table IV and Graph 4. This may be explained on the basis that the main ingredients which are present in Rexidin Plus mouthwash are Chlorhexidine gluconate, Triclosan and Sodium monofluorophosphate. Chlorhexidine is a bis-biguanide which is effective against Gram-positive bacteria, Gram-negative bacteria and yeast. At relatively high concentration, chlorhexidine is bactericidal but at low concentration it is bacteriostatic. This was in accordance with the study conducted by Hennessey TD who found reduction in the streptococcus mutans count due to the antibacterial property of chlorhexidine (Hennessey, 1973). The positively charged chlorhexidine binds readily to the negatively charged microbial cell surface. This is followed by disorganization of cytoplasmic membrane. Low concentrations of chlorhexidine allow cytoplasmic constituents to leak out while a high concentration coagulates them. It inhibits the membrane ATPase and anaerobic process which resulted in the decrease in the amount of plaque accumulation.

This was in accordance with the study conducted by Rolla G who also found the inhibition of plaque with the chlorhexidine gluconate (Rolla, 1975). The presence of fluoride in this mouth rinse could have also contributed to the reduction of S. mutans in plaque. The effects of fluoride on streptococcal cells are partly due to the inhibition of enolase, one of the series of gylcolytic enzymes. This was in accordance with the study conducted by Jenkins GN who found that the presence of fluoride in the mouth wash resulted in the reduction of streptococcus mutans count in plaque (Jenkins, 1959). Fluoride interacts with the metabolic and growth process in the bacteria by inhibiting the glycolytic enzyme which converts 2-P-glycerate to phosphoenol pyruvate (PEP). In the presence of fluoride, PEP inhibits the sugar transport in the PEP phosphotransfer system, causing bacterial cell death. This was in accordance with the study conducted by Hata S *et al*. (1990). In addition, fluoride can directly inhibit bacterial proton-translocating ATPase that is considered to partly contribute to the proton excertion out of the cells, leading to acidification of intracellular pH. The dissociation of unionized hydrofluoric acid into H+ and F– in the cells also promotes intracellular acidification. This can further reduce the bacterial metabolic activity.

The other ingredient present is triclosan, a broad-spectrum antimicrobial, having an anti-plaque potential. Triclosan (2,4,4'-trichloro 2'-hydroxydiphenyl ether) is used to increase the ability of mouthwashes to bind to the oral mucosa, and thus be available for longer periods of time. This was in accordance with the study conducted by Jenkins S *et al.* (1991) they concluded that the significant reduction in plaque *S. mutans* could be attributed to the synergistic effect of all three constituents chlorhexidine gluconate sodium monoflouro phosphate and triclosan. In the present study, when organic mouth wash (Hiora) was instructed to use after orthodontic appliance placement (Table 3 and Graph 3), it was observed that with the progression of time, there was a moderate decrease in the mean value of gingival index ,periodontal index ,gingival bleeding index and pocket depth.

The mean value was highest at To and lowest at T4 as shown in Table III. The mean value of streptococcus mutans (10^4 CFU/ml) in alcoholic mouthwash was found to be highest at T0 and was found to be least at T4 as shown in Table 4 and Graph 4. This might be due the fact that Hiora mouthwash is a herbal preparation, made from natural herbs with their beneficial properties like anticariogenic and antiplaque (due to S. persica which contains trimethyl amine, salvadorine, chlorides, high amounts of fluoride and silica, sulphur, vitamin C, small amounts of tannins, saponins, flavonoids and sterols. The alkaloid present in Salvadora persica (Miswak) is Salvadorine, which yields trimethylamine on hyrolytical cleavage. It exerts a bacteriocidal effect and stimulatory action on the gingiva. The silica present in the Miswak acts as an abrasive material to remove the stains giving the teeth whitness. The Sulphur compounds present in the miswak as shown by their pungent taste and smell have a bactericidal effect.

This was in accordance with the study conducted by Sadhan IR, Miswak (1990). Tannins also inhibit the action of glucosyl transferase thus reducing plaque and gingivitis. This mouthwash has antibiotic (due to the presence of Piper betle and Elettaria cardamomum) effect, antiinflammatory and immunity booster effect (due to the presence of Terminalia bellerica). Also, Mentha and Trachyspermum ammi which are natural flavouring agents. The results of our study is also in the favour of the study conducted by Babu S *et al* who also reported that the Hiora mouth rinse with aqueous Salvadora twigs extract causes significant reduction (84%) in the adherence of bacterial cells (Streptococcus mutans) to buccal epithelial cells and it was also reported that Piper betle, Elettaria cardamomum also significantly inhibited the growth of oral microflora and the reduction was up to 77% (Babu *et al*., 1996). When a multiple comparison was done between the mean value of streptococcus mutans at different intervals of time in different types of mouth wash, statistical significant difference was found organic mouthwash - alcoholic mouthwash and non alcoholic mouthwash –alcoholic mouthwash during the T3 and T4 as shown in Table 5 and Table 6.

The clinical and microbiologic effects of both the mouthrinses being comparable, leaves only one area where the comparison between the two could be possibly relevant. It has been reported that long term use of chlorhexidine is limited by staining of teeth and taste alteration2. However, no such effect has been reported with herbal extracts. A herbal mouthwash is a non-alcoholic preparation, with no added sugar, no artificial preservatives, no artificial flavors and colors and absolutely no side-effects. Thus, the herbal mouthrinses may provide oral health benefits, and provide a natural alternative for those consumers who wish to avoid artificial sweeteners, chemicals and alcohol contained in either over-the-counter or prescription mouthrinse products.. Moreover, it can serve as a good alternative for patients with special needs as in case of Diabetics, Xerostomics, etc. Thus, these can be used as an adjunct to mechanical therapy for treating plaque induced gingivitis. Present study has an important impact in order to create an effective and inexpensive oral health intervention for low socio-economic communities. However, this study was a short-term study so long-term studies are required with larger sample size.

Conclusion

The following conclusions drawn from the study were as follows

- Alcohol containing mouthwash (Listerine) showed a slight decrease in the level of the streptococcus mutans count where as non alcohol containing mouthwash (Rexidin Plus) showed a moderate decrease in the level of streptococcus mutans count during the early phase of orthodontic treatment.
- When comparison was done between different types of mouthwash, it was found that both organic mouthwash (Hiora) and non alcoholic mouthwash (Rexidin Plus) showed comparable clinical and microbiological results.

Organic mouthwash (Hiora) was found to be a potent plaque inhibitor, and were preferred by the patients for its taste, convenience of use and taste duration in their mouth after rinsing as compared to non alcoholic mouthwash (Rexidin Plus) which showed tooth discolouration, unpleasant tatse and burning sensations. Therefore, it is recommended to use organic mouthwash (Hiora) as compared to the Rexidin Plus mouthwash as it reduces the level of streptococcus mutan count and does not have any side effects.

REFERENCES

Shankar RP , Mahalakshmi K, Murali R.V , Padmavathy K, Balasubramanian S. Evaluation of Streptococcus Mutans Biofilm Retention by Three Different Types of Ligations on Orthodontic Brackets: An InVitro Scanning Electron Microscopic Study Effect of bracket types. *IOSR-JDMS 2014;13(6):70-74.*

Uzumer FD, Kaygisiz E, Cankaya ZT. Effect of bracket types on microbial colonization and periodontal status. *Angle Orthod 2014;84:1062-7.*

Antony VV, Khan R. Investigation of the Periodontal and Microbiological Status of Patients Undergoing Fixed Orthodontic Therapy. *IOSR-JDMS 2013;7(4):80-85.*

O'Reilly MM, Featherstone JDB. Demineralization and remineralization around orthodontic appliances: An in vivo study. *Am J Orthodontics 1987;92(1):33-40.*

Sukontapatipark W, Mohammad AE , Nils J, Thunold K, Selvig KA Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod 2001;23:475–484.*

Rosenbloom R G, Tinanoff N. Salivary Streptococcus mutans levels in patients before, during, and after orthodontic treatment. *Am J Orthod Dentofac Orthop 1991;100:35-7* .

Al-Mudallal NHA, Al-Jumaily EFA, Muhimen NAA, Al-Shaibany ABD Isolation And Identification of Mutan's Streptococci Bacteria From Human Dental Plaque Samples. *J Al-Nahrain Uni 2008;11(3):98-105.*

Fine D H, Letizia J, Mandel I D. The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. *J Clin Periodontology 1985;12:660-6.*

Kumar R *et al* Microbial Effect Of Different Mouthwashes An Patients Undergoing Fixed Orthodontic Treatment. *J Ind Orthod Soc 2006;39:139-146.*

Santos A: Evidence-based control of plaque and gingivitis. *J Clin Periodontal 2003;30 (5):13–16.*

Kulkarni VV, Damle SG Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the mutans streptococci count in saliva : An in vivo study. *J Indian Soc Pedo Prev Dent 2003;21(3): 98-104.*

Fine D H, Markowitz K, Furgang D *et al*. Effect of an essential oil-containing antimicrobial mouthrinse on specific plaque bacteria in vivo . *J Clin Periodontology 2007;34:652–7.*

Eley B. M. Antibacterial agents in the control of supragingival plaque —a review. *British Dental J 1999; 186:286–96.*

Moran J, Addy M, Kohut B *et al.* Efficacy of Mouthrinses in Inhibiting the Development of Supragingival Plaque Over a 4-Day Period of No Oral Hygiene. *J Periodontol 1994;65:904-7.*

Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol 1967;38 Suppl: 610-6.*

Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J 1975;25:229-35.*

Gjermo P, Baastad KL, Rolla G The plaque-inhibiting capacity of 11 antibacterial compourids. *J. periodont. Res. 1970; 5: 102-109.*

Hennessey TD Some antibacterial properties of chlorhexidine. *J Periodont Res 1973;8(12):61–67.*

Rolla G, Melsen V. On the mechanism of plaque inhibition of chlorhexidine. J Periodont Res. *1975;54:B57–62.*

Jenkins GN. Review of fluoride research since 1959. Arch Oral Biol. *1999;44:985–92.*

Hata S, Iwami Y, Kamiyama K, Yamada T. Biochemical mechanisms of enhanced inhibition of fluoride on the anaerobic sugar metabolism by Streptococcus sanguis. J Dent Res. *1990;69:1244–7.*

Jenkins S, Addy M, Newcombe R. Triclosan and sodium lauryl sulphate mouthwashes (I): Effects on salivary bacterial counts. *J Clin Periodontol. 1991;18:140–4.*

Miswak J. G. toothbrushes that grow on trees. Todays - FDA. 1990; 2: 60.

Babu S, Bhat RV, Kumar PU, Sasikaran B, Rao KV, Aruna P, Reddy PR. *A comparative clinico-pathological study of oral submucous fibrosis in habitual chewers of pan masala and betel quid. J Toxicol Clin Toxicol , 34:317322,(1996).*

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