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A comparative study of the anti-microbial efficacy of cetylpyridium chloride mouthwash and essential oil mouthwash in patients undergoing fixed orthodontic treatment – A randomised control trial

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Abstract

Background and Objectives: The control of supragingival plaque by means of mechanical devices such as toothbrushes, dental floss or interdental brushes is a key element in the prevention of caries and periodontal diseases. However, the presence of orthodontic fixed appliances makes this mechanical control more difficult thereby facilitating plaque retention, gingivitis and initial caries or white spot lesions. A common strategy to improve mechanical plaque removal in these patients is the addition of a chemical antimicrobial agent with anti-plaque or anti-caries activity in dentifrices, mouth rinses or both. The effectiveness of different active ingredients, such as Chlorhexidine (CHX), Essential oils, Amine/Stannous fluoride or Sanguinarine in the form of mouth rinses, toothpastes or gels have been evaluated in clinical studies. The use of Cetylpyridium chloride (CPC) has gained attention recently due to its improved bioavailability or increased concentration, which has shown increased clinical benefits and limited adverse effects such as tooth staining, gingival irritation and ulcers. CPC has been demonstrated in a number of clinical trials to reduce plaque formation and gingivitis.

Methodology: The study consisted of 60 subjects (15-25 years of age) who underwent fixed Orthodontic treatment in the department of Orthodontics and Dentofacial Orthopaedics after obtaining ethical clearance from the Institutional Review Board (MINDS/ PG- ETHICAL/ 005/ 2019-20). The subjects were

categorised into two groups (Group A and Group B) containing 30 subjects each. The subjects in Group A were given an Essential oil mouth rinse (Listerine Original) while the subjects in Group B received a Cetylpyridium chloride-based mouth rinse (VITIS Orthodontic).

The participants of the study were randomised using computer generated block randomisation. Full mouth oral prophylaxis was performed and oral hygiene instructions were given. Bonding of orthodontic appliance was then done in these patients. The baseline plaque samples (before introduction of mouthwash) were collected at the end of 4 weeks from the start of fixed orthodontic treatment. The patients were asked to rinse their mouth using 10ml of the given mouthwash after brushing twice daily. The plaque samples after the introduction of mouthwash were again collected at the end of 8 weeks and 12 weeks. The patients were asked to maintain a compliance diary which was evaluated at each visit. The plaque samples were collected using sterile paper points. The samples were then transported to the institute's Microbiology department in sterile containers using Thioglycolate transport medium. The samples were cultivated in blood agar in agar plates. The organisms were incubated at 37°C for 24 hours. The number of colonies in each plate was calculated using the formula: c = n / (s x d) where c = CFU/ml, n = number of colonies, d = dilution factor and s = volume transferred to plate.

Results: The CPC rinse showed significant antimicrobial benefits in vivo, reducing microbial levels by 68.4% which was 16.6% higher than the Listerine group (51.8%). The presence of additional ingredients such as allantoin (promotes cell proliferation), aloe vera (used to treat oral mucositis and aphthous stomatitis) and sodium fluoride (anti-caries agent) in the CPC mouth rinse could have been the reason for the superior antimicrobial activity when compared with the Listerine group. Additionally, the lack of alcohol in the formulation makes it a suitable adjunctive therapy for a broad spectrum of patients.

Keywords: Plaque, Mouthwash, Essential Oil, Listerine, Cetylpyridium Chloride, VITIS orthodontic mouthwash, Fixed orthodontic appliance.

Introduction

The control of supragingival biofilm by means of mechanical devices, such as toothbrushes, dental floss, or interdental brushes, is a key element in the prevention of caries and periodontal diseases. The presence of orthodontic fixed appliances, however, makes this mechanical control more difficult, facilitating plaque retention, gingivitis and initial caries or white spots lesions.[1] Enhanced plaque accumulation may also be associated with an increase in the colonization of periodontal pathogens, such as Prevotella intermedia.[2]

Moreover, many orthodontic patients, especially children and adolescents, fail to floss because they find this procedure time consuming and tedious in the presence of orthodontic arch wires.[3] Studies assessing compliance in these patients have reported that their level of cooperation varies considerably depending on their age and gender, personality, perception of the malocclusion, parental influence, and socioeconomic factors.[4]

As a result of these facts, adolescents or young adults wearing fixed orthodontic appliances tend to accumulate more plaque and hence, they are at a higher risk of developing gingivitis. A common strategy to improve mechanical plaque removal in these patients is the addition of a chemical antimicrobial agent with anti-plaque or anti-caries activity in dentifrices, mouth rinses, or both.[5] Ideally, these agents should have antimicrobial

activity, for reducing plaque accumulation and gingival inflammation[6]; anti-caries activity for decreasing the decalcifications usually occurring during orthodontic treatment and thus preventing the development of caries or white spots and anti-inflammatory activity to help in the healing of traumatic mucosal injuries caused by the appliances.[7]

Since these products are aimed for long-term use compliance is a critical factor and therefore, they should have a pleasant flavour and lack of side effects, such as staining or de-bonding of the braces and bands. The effectiveness of different active ingredients, such as chlorhexidine (CHX), essential oils, amine/stannous fluoride, or sanguinarine in the form of mouth rinses, tooth pastes, or gels, has been evaluated in clinical studies. Most of these clinical studies have reported significant benefits in the adjunctive use of these products, although the magnitude of these reported benefits might not have a clear clinical relevance. In addition, the use of some of the formulations was associated with adverse effects (such as staining with the use of CHX).[8-13]

The bactericidal efficacy of Listerine (Pfizer Consumer Healthcare, Morris Plains, NJ), the essential oil–containing mouth rinse, has long been recognized. The clinical benefits associated with the bactericidal activity of Listerine include prevention and reduction of supragingival plaque and gingivitis, decreased intrinsic oral malodour and a significant decrease in viable bacteria contained in the aerosols that are generated during dental procedures.[14-18]

In recent years, the use of the chemical antimicrobial agent cetylpyridinium chloride (CPC) has attracted some attention, due to the advent of formulations with improved bioavailability or increased concentration, which have shown increased clinical benefits and since their adverse effects are very limited (tooth staining, ulcers, gingival irritation), its use may be indicated for longer periods.[19,20] CPC has demonstrated in a number of clinical trials to reduce plaque formation and gingivitis. When used as a mouth rinse, a systematic review has shown heterogeneous results, although a significant effect on plaque has been demonstrated at different concentrations, such as 0.07 or 0.05 per cent.[21-26]

Recently, a new CPC-based mouth rinse and toothpaste were specifically formulated and marketed for orthodontic patients (VITIS Orthodontic, Dent aid, Cerdanyola, Spain). This formulation includes other active ingredients, such as allantoin, aloe vera, and sodium fluoride. Allantoin is widely used in dermatology since it promotes cell proliferation and stimulates epithelisation.[27] In dentistry, it has been added in formulations for the treatment of aphthous stomatitis. Similarly, aloe vera has been added in formulations for the treatment of oral mucositis in cancer patients and for aphthous stomatitis. This new formulation, aimed to be used by orthodontic patients, has not been evaluated in home use randomised clinical trials.[28]

It was, therefore, the objective of this investigation to assess and compare the anti-microbial efficacy of Cetylpyridium chloride-based mouth rinse and a standard Essential oil mouth rinse in patients undergoing fixed Orthodontic treatment, as an adjunct to mechanical plaque control.

Aim

The aim of this study is to assess and compare the anti-microbial efficacy of Cetylpyridium chloride-based mouth rinse and a standard Essential oil mouth rinse in patients undergoing fixed Orthodontic treatment, as an adjunct to mechanical plaque control.

Materials and Methods

A consecutive sample of subjects undergoing treatment with fixed orthodontic appliances was screened at the Department of Orthodontics in the authors' institution. During the screening visit, subjects were examined for fulfilment of the inclusion criteria and received a comprehensive explanation on the aims of the investigation. They were then asked to participate by signing an informed consent previously approved by the Ethical Review Committee. (MINDS/ PG- ETHICAL / 005/ 2019- 20)

Inclusion criteria

Patients within the age group of 15-25 years undergoing fixed orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics.

Exclusion criteria

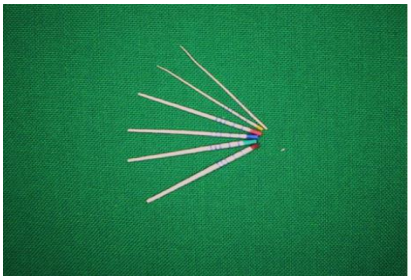
Patients with systemic illnesses, patients already using mouth rinses. Participants were excluded in presence of periodontitis (clinical attachment loss > 4 mm) or gingival overgrowth (pseudo pockets > 4 mm). Subjects were also excluded if they showed evidence of dental negligence.

Study design

The study was designed as a randomized, parallel, double-blinded, 3-month clinical trial. Participants and those assessing the outcomes were blinded to group assignment. **The following visits were scheduled**

At the screening visit, all recruited subjects had a professional prophylaxis followed by specific oral hygiene instructions. Bonding of the orthodontic brackets was then done (Ormco Mini Diamond) using light cured adhesives (Ormco Enlight). They were then asked to report after 4 weeks for the baseline visit.

During the baseline visit, the supragingival plaque samples were collected from around the orthodontic brackets using sterile paper points (Dentsply Protaper) (Fig.1). Samples were taken from the mesial-buccal site of upper first molars and the distal-buccal site of lower lateral incisors. Before the insertion of the paper points, sites were isolated with cotton rolls to avoid saliva contamination and the area was dried with an air syringe.[29] The collected samples were then transferred to a screw top vial (Fig.5) containing 1.5 ml of fluid Thioglycollate broth (Fig.2) transport medium (HI Media).



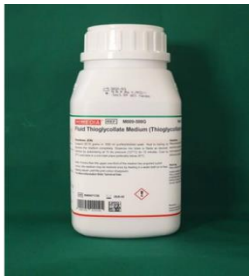


Fig 1: Paper points used for plaque collection

Fig 2: Thioglycollate transport medium

At the next visit, each participant was randomly assigned by the study supervisor to one of the study groups (Group A and Group B) through a computer-generated list (randomizer.org). Block size was not disclosed to ensure concealment. Each subject was given a unique number, which was associated with the assigned product. The subjects in Group A were given an Essential oil mouth rinse (Listerine Original) while the subjects in Group B received a Cetylpyridium chloride-based mouth rinse (VITIS Orthodontic) (Fig.3 & 4). The patients were then asked to rinse their mouth using 10ml of the given mouthwash after brushing twice daily. Patients were instructed to fill in a compliance diary where the home use of the products was recorded.

Fig.3,4: Essential oil, Cetylpyridium chloride

The plaque samples collected were then transported to the Department of Microbiology in sterile containers. The samples were cultivated in blood agar in agar plates (HI Media). The organisms were incubated at 37°C for 24 hours (Fig.6a). The number of colonies in each plate was estimated using a digital colony counter. During the next visit (at the end of 8 weeks), the plaque samples were once again collected and subjected to colony count similar to the previous visit.

At this visit, the compliance charts were collected and verified if the patients were adhering to the given guidelines. The patients were then reinforced to continue using the mouthwash for another month. At the final 3-month visit (at the end of 12 weeks), clinical parameters were evaluated and microbiological samples were taken for the final time to evaluate the colony count.

Fig 5: Plaque collected in screw top vial containing Thioglycollate transport broth

The patients were also each given a compliance form which they were asked to fill after using the mouthwash each time. These forms were evaluated at each visit and the patients were encouraged to continue using the mouthwash regularly and fill the compliance forms diligently until the study period was over.

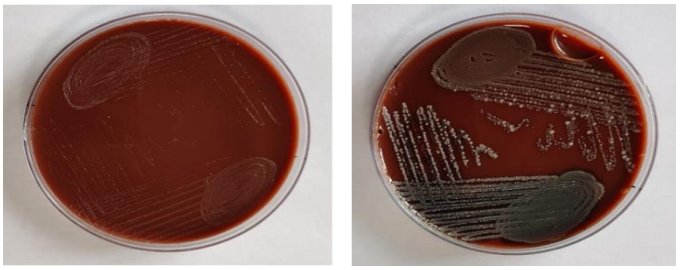


Fig 6a: Incubation of sample in blood agar; the picture on the right shows growth of micro-organisms after 24 hours

Results

Demographic data

Table 1: Mean age and Gender distribution of Group A

Gender	Sample size	Mean	SD
Male	10	20.00	2.98
Female	20	19.55	2.87
Total	30	19.70	2.87

Graph 1:

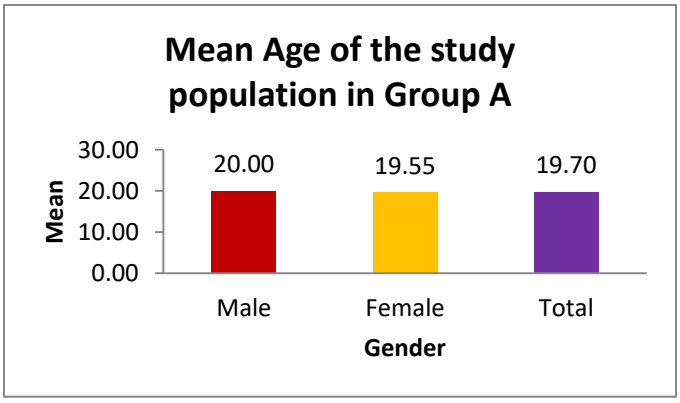
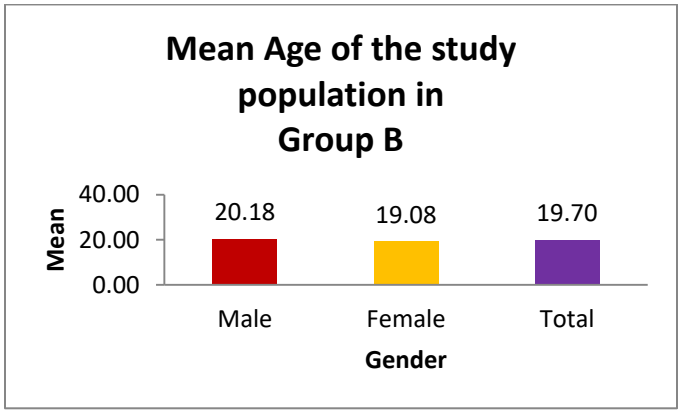


Table 2: Mean age and Gender distribution of Group B

Gender	Sample size	Mean	SD
Male	17	20.18	2.96
Female	13	19.08	3.20
Total	30	19.70	3.06

Graph 2:



Observations

The mean age of the subjects in both groups was 19 years of age. When it came to sex distribution, group A had more female patients when compared to male patients and this was the opposite in the case of group B.

Microbiological outcomes

Table 3: Intra-group comparison of Group A (Among the follow-ups)

Visit	Min	Max	Mean	SD	P value
Baseline Visit	1047128.00	5244359.00	2874028.80	1224871.95	<0.001*
2nd visit	523564.00	2622179.00	1437014.13	612435.95	
3rd visit	517456.00	2597546.00	1386524.17	590311.65	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level

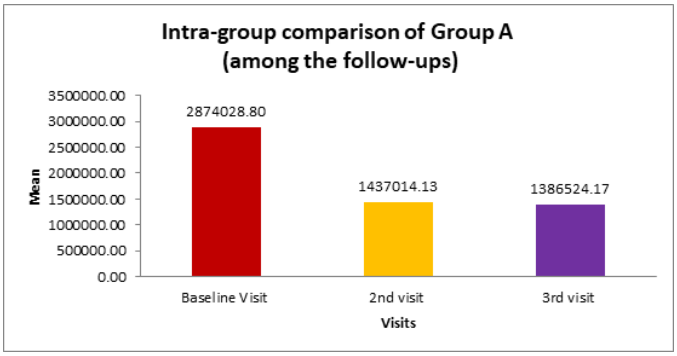
Table 4: Intra-group comparison of Group A (Between the follow-ups)

Visit	Mean	SD	Mean difference	% of mean reduction	P value
Baseline	2874028.80	1224871.95	-1437014.67	-50.0	<0.001*
2nd visit	1437014.13	612435.95			
Baseline Visit	2874028.80	1224871.95	-1487504.63	-51.8	<0.001*
3rd visit	1386524.17	590311.65			
2nd visit	1437014.13	612435.95	-50489.96	-3.5	<0.001*
3rd visit	1386524.17	590311.65			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 3:



Observations: The overall reduction in the colony count levels observed in group A was 51.8%.

Table 5: Intra-group comparison of Group B (Among the follow-ups)

Visit	Min	Max	Mean	SD	P vale
Baseline Visit	1071519.00	5688436.00	3140496.30	1368448.74	<0.001*
2nd visit	357173.00	1896145.00	1046831.80	456149.53	
3rd visit	351782.00	1824855.00	993243.60	435106.86	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level.

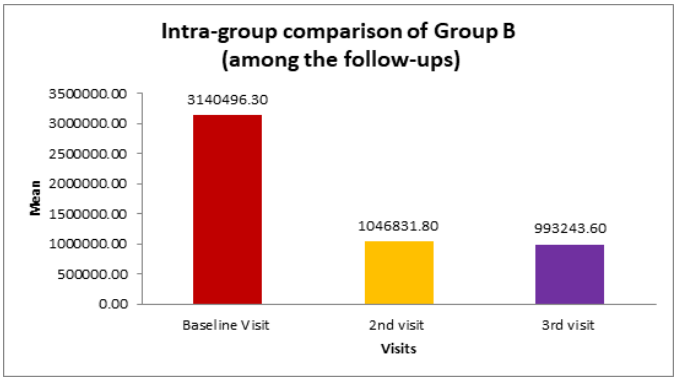
Table 6: Intra-group comparison of Group B (Between the follow-ups)

Visit	Mean	SD	Mean difference	% Of mean reduction	P value
Baseline Visit	3140496.30	1368448.74	-2093664.50	-66.7	<0.001*
2nd visit	1046831.80	456149.53			
Baseline Visit	3140496.30	1368448.74	-2147252.70	-68.4	<0.001*
3rd visit	993243.60	435106.86			
2nd visit	1046831.80	456149.53	-53588.20	-5.1	<0.001*
3rd visit	993243.60	435106.86			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 4:



Observations

The overall reduction in the colony count levels observed in group A was 68.4%.

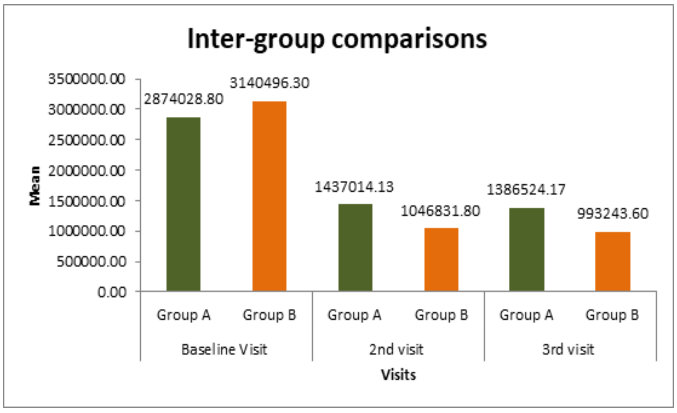
Table 7: Inter-group comparisons

Visit	Groups	Mean	SD	Mean difference	P value
Baseline Visit	Group A	2874028.80	1224871.95	266467.50	0.464 NS
	Group B	3140496.30	1368448.74		
2nd visit	Group A	1437014.13	612435.95	-390182.33	0.017 S
	Group B	1046831.80	456149.53		
3rd visit	Group A	1386524.17	590311.65	-393280.57	0.009 S
	Group B	993243.60	435106.86		

Statistical Analysis: Mann-Whitney U test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 5:



Observations

Inter-group comparisons performed by Mann-Whitney U test found that the colony count levels were comparatively lesser in group B when compared to group A. The overall reduction in the colony levels was 68.4% in group B when compared to the 51.8% in group A.

Table 8: Intra-group comparison of Group A males (Among the follow-ups)

Visit	Min	Max	Mean	SD	P value
Baseline Visit	1258925.00	4786300.00	2967674.10	1303186.20	<0.001*
2nd visit	629462.00	2393150.00	1483836.80	651593.12	
3rd visit	615875.00	2263280.00	1434818.50	607052.54	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level

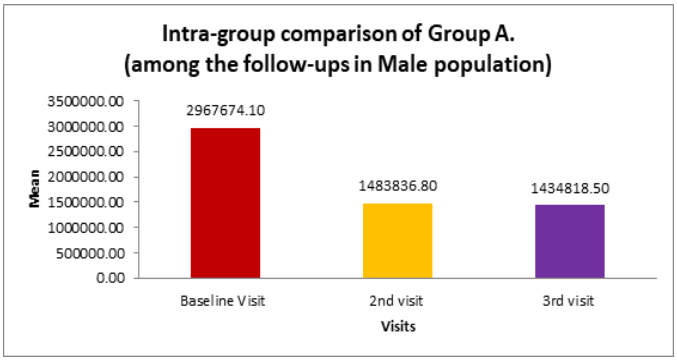
Table 9: Intra-group comparison of Group A males (Between the follow-ups)

Visit	Mean	SD	Mean difference	% Of mean decrease	P value
Baseline Visit	2967674.10	1303186.20	-1483837.3	-50.0	0.005*
2nd visit	1483836.80	651593.12			
Baseline Visit	2967674.10	1303186.20	-1532855.6	-51.7	0.005*
3rd visit	1434818.50	607052.54			
2nd visit	1483836.80	651593.12	-49018.30	-3.3	0.005*
3rd visit	1434818.50	607052.54			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 6:



Observations

The male population in group A showed a 51.7% reduction in the overall colony count levels at the end of 3 months.

Table 10: Intra-group comparison of Group B males (Among the follow-ups)

Visit	Min	Max	Mean	SD	P value
Baseline Visit	1071519.00	5495408.00	2890261.41	1404785.85	<0.001*
2nd visit	357173.00	1831802.00	963420.18	468261.85	
3rd visit	351782.00	1772546.00	916932.59	443187.18	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level.

Table 11: Intra-group comparison of Group B males (Between the follow-ups)

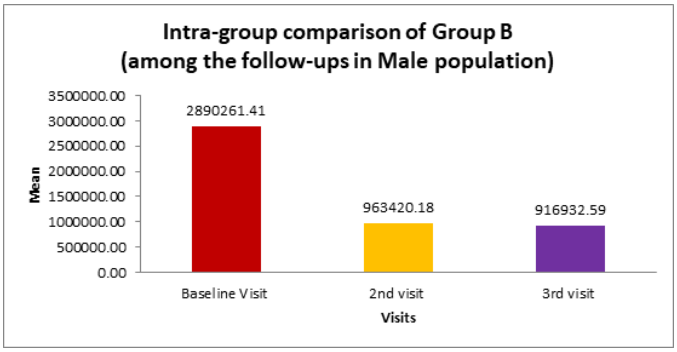
Visit	Mean	SD	Mean difference	% of mean decrease	P value
Baseline Visit	2890261.41	1404785.85	-1926841.2	-66.7	<0.001*

2nd visit	9634 20.18	4682 61.85			
Baseline Visit	2890 261.41	1404 785.85	-197332 8.8	-68.3	<0.001*
3rd visit	9169 32.59	4431 87.18			
2nd visit	9634 20.18	4682 61.85	-46487.59	-4.8	<0.001*
3rd visit	9169 32.59	4431 87.18			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 7:



Observations

The male population in group B showed a 68.3% reduction in the overall colony count levels at the end of 3 months.

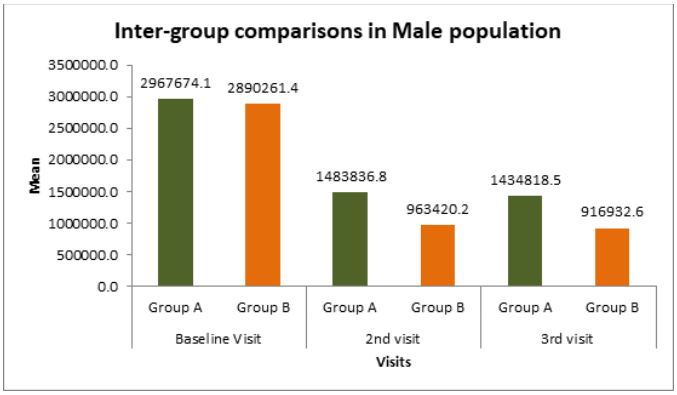
Table 12: Inter-group comparisons in male population

Visit	Groups	Mean	SD	Mean difference	P value
Baseline Visit	Group A	29676 74.10	1303 186.20	-77412.69	0.900
	Group B	28902 61.41	1404 785.85		
2nd visit	Group A	14838 36.80	6515 93.12	-520416.6	0.045*
	Group B	96342 0.18	4682 61.85		
3rd visit	Group A	14348 18.50	6070 52.54	-517885.9	0.040*
	Group B	91693 2.59	4431 87.18		

Statistical Analysis: Mann-Whitney U test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 8:



Observations

Between the two groups, the male subjects in group B showed a comparatively higher percentage of reduction in the colony count levels during the 2nd and 3rd visits.

Table 13: Intra-group comparison of Group A females (Among the follow-ups)

Visit	Min	Max	Mean	SD	P value
Baseline Visit	104712 8.00	524435 9.00	282720 6.15	121596 4.81	<0.001*
2nd	523564	262217	141360	607982	

visit	.00	9.00	2.80	.35	
3rd visit	517456.00	259754 6.00	136237 7.00	596214.69	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level.

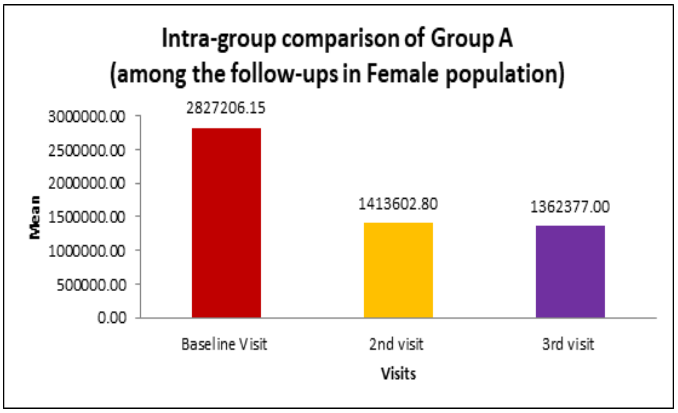
Table 14: Intra-group comparison of Group A females (Between the follow-ups)

Visit	Mean	SD	Mean difference	% Of mean decrease	P value
Baseline Visit	282720 6.15	121596 4.81	-141360 3.3	-50.0	<0.001*
2nd visit	141360 2.80	607982.35			
Baseline Visit	282720 6.15	121596 4.81	-146482 9.6	-51.8	<0.001*
3rd visit	136237 7.00	596214.69			
2nd visit	141360 2.80	607982.35	-51225.80	-3.6	<0.001*
3rd visit	136237 7.00	596214.69			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 9:



Observations

The female patients in group A showed a 51.8% reduction in the overall colony count levels at the end of 3 months.

Table 15: Intra-group comparison of Group B females (Among the follow-ups)

Visit	Min	Max	Mean	SD	P value
Baseline Visit	165958 6.00	568843 6.00	346772 6.54	129985 9.03	<0.001*
2nd visit	553195.00	189614 5.00	115590 8.54	433286.37	
3rd visit	506981.00	182485 5.00	109303 4.92	420230.90	

Statistical Analysis: Friedman's test. *P<0.05. i.e., the P value is significant at the 0.05 level.

Table 16: Intra-group comparison of Group B females (Between the follow-ups)

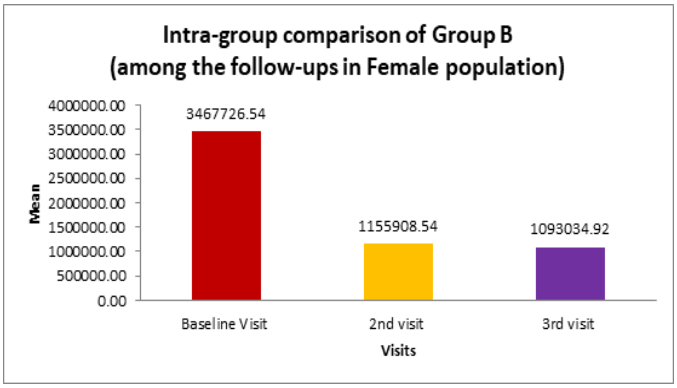
Visit	Mean	SD	Mean difference	% of mean decrease	P value
Baseline Visit	346 772 6.54	12998 59.03	-2311818	-66.7	0.001*
2nd visit	115 590 8.54	43328 6.37			

Baseline Visit	3467726.54	1299859.03	-2374691.6	-68.5	0.001*
3rd visit	1093034.92	420230.90			
2nd visit	1155908.54	433286.36	-62873.62	-5.4	0.001*
3rd visit	1093034.92	420230.90			

Statistical Analysis: Wilcoxon Signed ranks test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 10:



Observations

The female patients in group B showed a 68.5% reduction in the overall colony count levels at the end of 3 months.

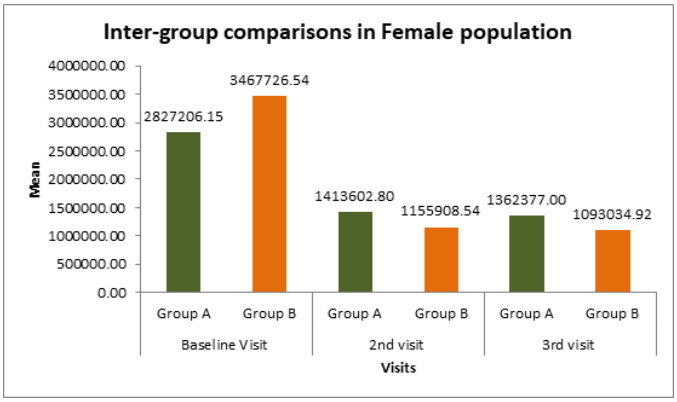
Table 17: Inter-group comparisons in female population

Visit	Groups	Mean	SD	Mean difference	P value
Baseline Visit	Group A	2827206.15	1215964.81	640520.39	0.197
	Group B	3467726.54	1299859.03		
2nd visit	Group A	1413602.80	607982.35	-257694.26	0.302
	Group B	1155908.54	433286.37		
3rd visit	Group A	1362377.00	596214.69	-269342.08	0.238
	Group B	1093034.92	420230.90		

Statistical Analysis: Mann-Whitney U test.

*P<0.05. i.e., the P value is significant at the 0.05 level.

Graph 11:



Observations

It was noted that the female subjects in group B showed better antimicrobial properties during the 2nd and 3rd visits when compared with the female population in group A.

Discussion

In the present study, the adjunctive use of mouth rinses and dentifrice had a significant effect in the reduction of microbial levels in both the groups. The use of this adjunctive treatment was not associated with an increase in tooth staining; moreover the compliance during this 3-month period with the study protocol, in both groups, was exceptional. In addition, the test products were able to control and even reduce periodontal pathogen levels, without an overgrowth of opportunistic species.

Out of the 30 patients in Group A, 10 were male and 20 were female patients with a mean age of 19 years. Group

B consisted of 17 male patients and 13 female patients with a mean age of 19 years. (Table 1 & 2)

The initial colony count levels at the baseline visit ranged between 1047128 CFU to 5244359 CFU with a mean of 2874028.80 and standard deviation of 1224871.95 for the Listerine group (Group A). During the 2nd visit i.e. after 8 weeks, a 50% reduction was observed in the colony count levels with a mean of 1437014.13 and SD of 612435.95. During the final 12th week visit, a 3.5% reduction in the colony levels was noted with a mean value of 1386524.17 and SD of 590311.65. The 3.5% reduction between the 2nd and 3rd visits was negligible when compared to 50% reduction observed between the baseline visit and the 2nd visit. The overall reduction in the colony levels was found to be 51.8% between the baseline visit and the 3rd visit with a p value of <0.001 (statistically significant). (Table 3 & 4)

Similarly for the CPC group (Group B), the colony count levels ranged between 1071519 CFU to 5688436 CFU with a mean of 3140496.30 and SD of 1368448.74 during the baseline visit. After 8 weeks the colony count levels ranged between 357173 CFU to 1896145 CFU with a mean of 1046831.80 and SD of 456149.53. A 66.7% reduction in the microbial levels was observed between the baseline visit and the 2nd visit. During the 12-week visit, a 5.1% reduction was noted with a mean value of 993243.60 and SD of 435106.86. The overall reduction between the 1st and final visit was found to be 68.4% with a p value of <0.001 which is statistically significant. (Table 5 & 6)

Inter-group comparisons performed by Mann-Whitney U test found that the colony count levels were comparatively lesser in group B when compared to group A. The overall reduction in the colony levels was 68.4% in group B as compared to the 51.8% in group A. Also, the patients using CPC mouthwash showed superior antimicrobial activity between all three visits when compared to the patients using Listerine mouthwash. The mean difference between the groups was found to be 266467.50 at the baseline visit with a p value of 0.464 (statistically insignificant). During the 2nd visit, the p value was observed to be 0.017 (statistically significant) and the p value during the 3rd visit was noted as 0.009 (statistically significant). (Table 7)

Inter-sex comparisons in group A showed that the mean colony count levels in males were 2967674.10, 1483836.80 and 1434818.50 during the 1st, 2nd and 3rd visit respectively with an overall reduction of 51.7% (Table 8 & 9). The female patients in group A showed a mean of 2827206.15, 1413602.80 and 1362377.00 during their subsequent visits. The overall reduction in colony count levels was noted to be 51.8% (Table 13 & 14) which was similar to that of the male subjects. The difference in the overall percentage reduction between the male and female patients in group A is considered to be statistically insignificant.

In the CPC group, the mean colony count levels in male patients were 2890261.41, 963420.18 and 916932.59 during the 1st, 2nd and 3rd visits respectively. The overall reduction in colony levels was found to be 68.3% (Table 10 & 11). The mean colony count levels for the female subjects in group B were 3467726.54, 1155908.54 and 1093034.92 during their subsequent visits (Table 15 & 16). The overall percentage reduction was found to be 68.5% which was similar to that of the male patients in group B. One additional thing to note is that the female patients showed comparatively higher colony count levels during the baseline visit when compared to the males. But at the end of 3 months, the

overall reduction in colony count levels was similar in both the sexes.

There were no significant statistical differences between the two groups when both the sexes were compared with each other. (Table 12 & 17) Compliance with the use of the tested products was evaluated by compliance forms. All the patients in both the groups filled their compliance forms regularly and used the given mouthwashes without fail. It is well documented in literature that brushing and flossing are the 'gold standard' procedures for controlling bacterial plaque. However, based on results derived from several clinical trials, the ADA has recommended a mouth rinse containing EO (Listerine) as an adjunct to routine mechanical oral hygiene measures. Several clinical studies have demonstrated that EO-containing mouth rinses can combat harmful bacteria and improve oral health.

The mechanism of action of Listerine involves bacterial cell wall destruction, bacterial enzymatic inhibition, and extraction of bacterial lipopolysaccharides. The clinical benefits associated with the bactericidal activity of Listerine include prevention and reduction of supragingival plaque and gingivitis, decreased intrinsic oral malodor and a significant decrease in viable bacteria contained in the aerosols that are generated during dental procedures.[14-16]

Four 6-month or longer controlled clinical trials have shown Listerine to be significantly effective in helping prevent the development of both supragingival plaque and gingivitis. Two microbiology studies have demonstrated that no resistant micro-organisms, opportunistic micro-organisms, or presumptive oral pathogens emerge as a result of long-term, daily Listerine use. Listerine is the first non-prescription mouth rinse to receive the Council on Dental Therapeutics Seal of Acceptance as safe and effective in helping to prevent and reduce supragingival plaque accumulation and gingivitis when used in a conscientiously applied program of oral hygiene and regular professional care.

A study was conducted to assess the irritation potential of an EO-containing mouth rinse (Listerine antiseptic) in a population with objectively documented xerostomia (hyposalivation) using an exaggerated-exposure clinical model. The oral irritation potential of the EO mouth rinse was minimal and oral mucosal abnormalities attributable to the test rinses were seen in only two subjects, both at the 7-day examination. These subjects were both using the EO mouth rinse. The abnormalities consisted of an asymptomatic ''whitish slough" which was readily wiped off leaving a normal appearing, non-erythematous mucosa. In both subjects, the oral mucosa appeared normal at the 14-day examination.[30]

Recently, an alcohol-free oral rinse product (VITIS orthodontic) was specifically developed for patients undergoing orthodontic treatment. CPC has a long heritage of use as a broad-spectrum antimicrobial against oral bacteria. It was one of only three antimicrobial systems to be classified as safe and efficacious for the treatment of plaque-induced gingivitis, when formulated within a concentration range of 0.05 and 0.10%, by the FDA Plaque Subcommittee following a six-year review of over 40 active ingredients. The other two active ingredients were stannous fluoride and essential oils. CPC acts primarily by penetrating the cell membrane, which causes leakage of components in the cell, disruption of bacterial metabolism, inhibition of cell growth and finally cell death.[31,32]

In the current study, both Listerine and CPC mouthwash showed significant antimicrobial properties by reducing the colony count levels by 51.8% and 68.4%

respectively. This antibacterial activity translates into an in vivo benefit, as demonstrated by the 0.05% CPC rinse's inhibition of microbial growth in the clinical trial. The clinical study showed significant benefits for the CPC rinse relative to the Listerine group. Not only does the novel CPC rinse deliver therapeutic benefits, but the lack of alcohol in the formulation makes it suitable for a broad range of patients. Most over-the-counter rinses contain between 5-22% alcohol, primarily for formulation purposes to assist in the solubilisation of certain ingredients. The inclusion of alcohol limits their use among certain patient groups (e.g., children, diabetics, alcoholics, patients with xerostomia, members of certain religious faiths). The new CPC rinse also provides gingival health benefits without the burn of alcohol, which encourages patient compliance.

It is important to note common excipients added to commercial oral care formulations, such as surfactants, can diminish or even completely neutralize the antimicrobial activity of CPC.[33,34] Published data show formulations with high bioavailable CPC are associated with greater biological activity and also suggest these formulations would have a higher probability of showing clinical efficacy. The novel product has been formulated to have >76% CPC bioavailability, thereby fulfilling the requirements for a safe and efficacious CPC oral rinse for the treatment of plaque-induced gingivitis, as suggested by the FDA.

Summary and conclusion

It is well known that patients undergoing orthodontic therapy show higher plaque levels. The presence of orthodontic fixed appliances makes mechanical control more difficult, facilitating plaque retention, gingivitis and initial caries or white spots lesions. A common strategy to improve mechanical plaque removal in these patients is the addition of a chemical antimicrobial agent with anti-plaque or anti-caries activity in dentifrices, mouth rinses or both.

In the present study, the antimicrobial efficacy of two commercially available mouth rinses (Listerine and VITIS orthodontic) was evaluated over a three-month period and the following observations were noted;

1. Both Listerine and VITIS orthodontic mouthwashes showed significant antimicrobial activity over the three-month study period.
2. Listerine showed a 51.8% reduction in the colony count levels while VITIS orthodontic mouthwash showed a 68.4% reduction when compared with the baseline samples.
3. The reduction in the colony count levels between the 2nd and 3rd visits was very minimal. (3.5% in Group A and 5.1% in Group B)
4. There was no significant difference in the antimicrobial efficacy of the mouth rinses between sexes in both the groups.
5. Both groups showed good level of compliance throughout the study period
6. Neither groups showed any ill effects after using the mouthwashes over a three month period.

The use of antimicrobial mouth rinses in patients undergoing orthodontic therapy is highly encouraged as proved by the present study. Listerine being one of the most recognised brands globally has been prescribed by dentists for its highly efficacious anti-plaque and antimicrobial properties. It has certain advantages over the gold standard chlorhexidine containing mouthwashes, like being used for a prolonged time without causing staining of teeth or causing alterations in taste which are some of the common side effects of chlorhexidine mouthwashes. On the other hand, the no-alcohol, high bioavailable CPC rinse formulation demonstrated a wide spectrum of antimicrobial activity.

The CPC rinse showed significant antimicrobial benefits in vivo, reducing microbial levels by 68.4% which was 16.6% higher than the Listerine group (51.8%). The presence of additional ingredients such as allantoin (promotes cell proliferation), aloe vera (used to treat oral mucositis and aphthous stomatitis) and sodium fluoride (anti-caries agent) in the CPC mouth rinse could have been the reason for the superior antimicrobial activity when compared with the Listerine group. Additionally, the lack of alcohol in the formulation makes it a suitable adjunctive therapy for a broad spectrum of patients.

Since this was a short-term study (3 months), the patients were more likely to adhere to the provided instructions and hence resulted in significant antimicrobial activity of both the mouth rinses. More long-term clinical studies are required to further evaluate the merits and demerits of using mouthwashes as an adjunct to mechanical plaque control in patients undergoing fixed orthodontic therapy.

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