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Comparative Evaluation of Chlorhexidine Gluconate And Cetylpyridinium Chloride For Disinfection of Acrylic Plates of Removable Orthodontic Appliances - An In Vivo Study

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Abstract

Background: *The use of removable orthodontic appliances increases the microbial contamination of the oral cavity. The use of an apt antimicrobial agent is necessary to reduce cross contamination.*

Aim : *To evaluate and to compare the effectiveness of 0.12% chlorhexidine and 0.05% Cetylpyridinium chloride in the disinfection of acrylic plates of removable orthodontic appliances.*

Materials and Methods: *15 patients of age 6-12 years were selected for the study. Appliances were made from self-cure acrylic resin on cast models with retentive wire components. Patients were recalled, wire components were cut, and sprayed with tap water, 0.12% CHX, and 0.05% CPC, and sent for microbiological analysis every week. Three appliances were made for each patient. The microbial colonies adhering on the plates were counted and scored on a 3 point scale.*

Results - *With tap water 73.3% of the plates showed score 3, whereas 26.7% showed score 2. With 0.12% CHX 33.3% of the plates showed score 0, where as 60% of the plates showed score 1, and 6% of the plates showed score 2. With 0.05% CPC 6.6% of the plates showed score 0, whereas 60% of the plates showed score 1, and 33.3% showed score 2.*

Conclusion: *0.12% CHX showed better reduction of microbial colonies when compared to other two solutions used.*

Keywords: *Chlorhexidine gluconate, Cetylpyridinium Chloride, removable orthodontic appliances, Streptococcus Mutans*

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I. Introduction

Dental appliances used by patients are exposed to normal oral microbial flora that includes bacteria, viruses, and fungi. The use of removable orthodontic appliances for minor tooth movements, retention and myofunctional therapies results in greater biofilm accumulation on the retentive sites of the retentive, active components and, also the acrylic base plates. Dental personnel adjusting or repairing these appliances may therefore be at a risk of contracting infections from the appliances that have not been properly disinfected.[1]

Plaque control is fundamental in the control of caries and periodontitis. It has been shown that placing a fixed orthodontic appliance leads to both an increase in the levels and a change in the composition of dental plaque.[2,3] Many studies by Sakamaki and Bahn[4], Corbett et al[5], Scheie et al[6] have suggested that placing an orthodontic appliance leads to the creation of new retentive areas favoring the local growth of organisms, and specifically an increase in level of Streptococcus mutans.Because of the infectiousness of dental caries, reducing the levels of cariogenic microorganisms would prevent caries onset.[7] Therefore, antimicrobial agents have been advised for orthodontic patients to aid in the control of bacterial biofilm formation, because toothbrushes cannot completely remove microorganisms from critical retentive sites of orthodontic appliances.[8] Chlorhexidine gluconate (CHX) belongs to the biguanides chemical group. It is bactericidal, viricidal, and fungicidal, causing cell wall decomposition, leading to the loss of the cell's components.[9]Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound in some types of mouthwashes, toothpastes, lozenges, throat sprays, breath sprays, and nasal sprays. It is an antiseptic that kills bacteria and other microorganisms. It has been shown to be effective in preventing dental plaque and reducing gingivitis. It is capable of adsorbing to negatively charged bacterial cell membrane phosphates possibly disrupting the cell wall and increasing

permeability. Cetylpyridinium chloride was shown to be bactericidal to Gram positive bacteria but relatively ineffective against some Gram negative bacteria.[10]Several studies have shown that Cetylpyridinium chloride containing mouthwashes inhibit plaque formation [Holbeche et al (1978), Allen et al (1998)].[11,12] Even though comparative studies have been carried out with Chlorhexidine and Cetylpyridinium chloride, they were related to elderly people, who are at risk to develop periodontitis. But when children are concerned, Streptococcus mutans inhibition seems to be the most important function to evaluate the potency of antimicrobial solutions. The antimicrobial effect and range of Chlorhexidine has been proved in many studies.[9,13] But considering an alternative for Chlorhexidine, the range of antibacterial activity has been found similar in Cetylpyridinium chloride.[14] The efficacy of Cetylpyridinium chloride has been tested in in-vitro studies.[15,16,17] But not many studies have been conducted to prove its effectiveness in children.

The aims of the study were to evaluate the effectiveness of 0.05% Cetylpyridinium chloride and 0.12% Chlorhexidine gluconate for disinfection of acrylic plates of removable orthodontic appliances during orthodontic treatment, and to compare the effectiveness of 0.05% Cetylpyridinium chloride and 0.12% Chlorhexidine gluconate for disinfection of acrylic plates of removable orthodontic appliances during orthodontic treatment.

II. Materials And Methods

Fifteen patients of both sexes, aged between 6-12 years i.e. children with mixed dentition, were randomly selected and included in the study. Patient's whose permanent first molar has not erupted, those who were using antimicrobial mouthwash, those with systemic diseases and patients who had used antibiotics in the past three months were not included in the study. Base plates of orthodontic appliances were fabricated on cast models from each patient enrolled for the study. The base plates were fabricated from cold cure acrylic resin (DPI-RR), and the appliances were trimmed and polished. The patients were instructed to wear the appliance full time, including during sleep. It was also instructed that the appliance should be removed only during meal times. The children were instructed to brush their teeth thrice a day after meals and the parents were instructed to brush the appliance once daily, at bed time using the same tooth brush and dentifrice used by the children to brush their teeth.The children were recalled a week after the delivery of the appliance. The appliance was then disinfected using a randomized disinfection protocol. A new appliance was fabricated and the same procedure was carried out for another two weeks. For the randomized disinfection protocol, two antimicrobial solutions were used. Tap water was used as a control. The solutions were transferred to individual plastic trigger spray bottles.The wire components were cut off from acrylic base plates using a sterile wire cutter. The plate was maintained in a fixed position using tweezers and one test solution was sprayed on the entire surface of base plates, three times on each surface. Every week different test solutions were used. The sprayed acrylic plates were stored in sterile closed containers containing Bacitracin Streptococcus enrichment broth (HiMedia Laboratories) and were incubated for four days at 37^{o}C. The acrylic plates were gently withdrawn from the containers and rinsed in the broth. Streptococcus mutans colonies were counted and expressed according to a 3-point scale.

1. 0→ 0 colonies of Streptococcus mutans
2. 1→ 1-50 colonies of Streptococcus mutans
3. 2→ 51-100 colonies of Streptococcus mutans
4. 3→>100 colonies of Streptococcus mutans

III. Statistical Analysis

Kruskal-Wallis test was carried out. If P- value was less than 0.05, the null hypothesis was rejected and the alternate hypothesis was accepted. If there was a significant difference, multiple comparisons were carried out using Mann-Whitney test.

IV. Results

Fifteen patients were included in the study and 45 appliances were delivered to them. 53% were males and 47 % were females**.** The mean age of children included in this study was 9.4 years, among which selected males had a mean age of 9.63 years, and that of females was 9.14 years. **(Table-1)**

Table 1: Mean Age According To Gender

Group	Mean Age	Sd	Median	Min	Max
Males	9.63	2.00	8.5	8	12
Females	9.14	1.77	9.0	7	12
Overall	**9.40**	**1.84**	**9.0**	**7**	**12**

In the first week when tap water was used to spray the plates, 73.3% of the plates disinfected in the first week showed more than 100 colonies (score 3), whereas 26.7% of them showed about 50-100 colonies (score 2), and 0% of the plates showed 0-50 colonies (score 1) and 0 colonies (score 0). **(Fig-1)**

Fig – 1: orthodontic plate sprayed with tap water, and incubated

In the second week when 0.12% Chlorhexidine gluconate was used, 33.3% of the plates disinfected with 0.12% Chlorhexidine gluconate didn't show any microbial colonies after incubation, where as 60% of the plates showed 0-50 colonies (score 1), and 6% of the plates showed 50-100 colonies (score 2). **(Fig-2)**

Fig – 2: orthodontic plates sprayed with 0.12% CHX, and incubated

At the end of the third week, when 0.05% Cetylpyridinium chloride was used, 6.6% of the plates disinfected with 0.05% Cetylpyridinium chloride didn't show any colonies, whereas 60% of the plates disinfected on the third week showed 0-50 colonies (score 1), and 33.3% showed 50-100 colonies (score 2). **(Fig-3)(Table-2)**

Fig – 3 : orthodontic plates sprayed with 0.05% CPC and incubated

Table 2: Distribution of microbial scores in the three solutions

Solution	Microbial Colonies				Total
	No Microbial Colonies	0 To 50 Microbial Colonies	50 To 100 Microbial Colonies	>100 Microbial Colonies	
Sterile Water	0	0	4	11	15
Chx	5	9	1	0	15
Cpc	1	9	5	0	15
Total	**6**	**18**	**10**	**11**	**45**

Comparison of scores in the three solutions

The mean score for Streptococcus mutans colonies on the acrylic plate was found to be higher in control solution i.e. tap water. No inhibition of Streptococcus mutans colonies were found when sprayed with tap water. Higher scores were found when compared to plates disinfected with Chlorhexidine and Cetylpyridinium chloride. The mean score found, when the plates were disinfected with tap water was 2.73, with a SD of 0.46. The mean score when they were sprayed with Chlorhexidine was found to be 0.73 with a SD of 0.59. Similarly, the mean score for the third group i.e. when the plates were disinfected with Cetylpyridinium chloride was found to be 1.27 with a SD of 0.59. The difference in microbial scores between the three groups was found to be statistically significant (P<0.001). **(Table-3).**

Table 3: Comparison of microbial scores in the three solutions

Solution	Mean	Sd	Min	Max	Kruskal-Wallis Chi-Sq	P-Value
Sterile Water	2.73	0.46	2	3	31.250	<0.001*
Chx	0.73	0.59	0	2	31.250	<0.001*
Cpc	1.27	0.59	0	2	31.250	<0.001*

*denotes significant difference

Since statistically significant results were obtained when comparing the antimicrobial activity of the control and the test solution, it was necessary to compute the multiple comparisons. Thus Mann-Whitney test was carried out. The difference between the mean scores for tap water and CHX was 2 and that of tap water and CPC was 1.467, which gave a Z-value of -4.79 and -4.487 respectively and the differences were found to be statistically significant (P<0.001). Also, the mean difference in microbial scores between CHX and CPC was found to be -0.533 with a Z-value of -2.271. The difference in microbial scores when CHX and CPC were used was found to be statistically significant showing a P- value of 0.023 (P<0.05). **(Table – 4)**

Table 4: comparison of microbial score between pairs of groups

Solution (I)	Solution (Ii)	Mean Difference (I-Ii)	Z	P-Value
Sterile Water	**Chx**	2.000	-4.790	<0.001*
	Cpc	1.467	-4.487	<0.001*
Chx	**Cpc**	-0.533	-2.271	0.023*

* denotes significant difference

V. Discussion

Removable orthodontic appliances are constructed with stainless steel wires of variable diameters and self-curing poly methyl methacrylate-based resin that undergoes different monomer-polymer reactions.[18]It was reported that acrylic resin dentures worn by adults might harbor harmful pathogens in resin pores on outer and inner surfaces.[19] The findings of a study by Sukontapatipark W et al. (2001) showed that, regardless of the type of surface polishing, microorganisms might penetrate as deep as 1 to 2 μm into acrylic resin denture bases and remain viable. In addition, poly methyl methacrylate shows long-term water sorption due to water molecule diffusion, probably spreading the macromolecules out.[1,20] The surface imperfections on acrylic surfaces can range form microscopic fissures to defects visible to the naked eye and might not only weaken the resin structure but also facilitate the retention and adherence of microorganisms.[21] These microorganisms attack not only the acrylic surface, but also the dental enamel, thus altering the normal oral microbiota. Therefore, even if adequately polished, the acrylic base plates of removable orthodontic appliances are a hard, non-scaling surface for the adherence of Streptococcus mutans, which are aciduric and acidogenic microorganisms. This, and the retentive niches for food and bacteria to lodge during orthodontic appliance therapy contribute to local pH decrease, enamel demineralization, and caries onset.[22]In this study, microbial colonies were adhered to the surface of the acrylic surface at the end of the first week. The considerable retention and adherence of Streptococcus mutans on the surface of acrylic base-plates of removable orthodontic appliances were confirmed in this study because, the specimens sprayed with tap water (non-disinfected control) showed contamination

with many Streptococcus mutans colonies / biofilms, showing uncountable colonies which had to be scored for score 3. In a similar study conducted by Lessa et al[1] 82.3% of the plates showed score 3, 11.7% of the plates showed score 2, and 5.8% of the cases showed score 1, while in this study, 73.3% of the plates sprayed with tap water showed score 3, 26.7% of the plates showed score 2. Adherence of Streptococcus mutans colonies was seen to be similar with the above quoted study since a non-disinfecting control was used. An in-vitro study has been carried out regarding the adherence of Streptococcus mutans colonies on acrylic dentures and acrylic blocks by N. Okita et al[23], who used soft liners to check their antimicrobial activity which included acrylic resins too, they found that the Streptococcus mutans level adhesions had been found significantly greater whether acrylic resin was used or with the use of soft liners. Chlorhexidine (CHX) has been established as the most effective chemical plaque-control compound.[24,25] It interferes with biofilm formation and prevent the growth processes.[26,27] Evidences suggested that bacterial phenotypes may be modified when the organisms change from a planktonic to a sessile state.[28] Pratten et al. demonstrated that 0.2% CHX gluconate had little effect on the viability of established biofilms in vitro after pulsing twice daily for 4 days.[29] Another in vitro study conducted by Zaira Aurite et al. demonstrated a small effect of CHX on developed biofilm viability.[30] Several clinical trials conducted by, Stoecken et al. demonstrated that CHX is effective in reducing the formation of dental plaque.[31]

Fabrı´cio B. Zanatta et al. (2007) studied the effect of 0.12% CHX and found out that it had little effect on already formed plaque. They concluded that there is a need of disruption of the plaque before the initiation of CHX mouth rinse.[32]It was shown that daily immersion of complete dentures in 0.2% or 2% Chlorhexidine gluconate might cause staining.[33] For this reason, in this study, Chlorhexidine gluconate was used in a 0.12% concentration as a test solution, as no evidence of staining of acrylic plates was found from previous literature.

In this study 33.3% of the plates didn't show any colonies, 60% of the plates gave score 1, and 6% gave score 2 respectively, which agrees with the study conducted by Lessa et al (2007).[1] where, 35.2%, 58,8%, and 5.8% of the plates disinfected with CHX showed score 0,1,2 respectively whereas none of them showed score 3. Cetylpyridinium chloride oral rinse (CPC) was formulated as an alcohol-free alternative to other anti-plaque and anti-gingivitis oral rinses. The mechanism of action of CPC on Streptococcus mutans is exhibited by its effect on cell surface hydrophobicity, that inhibits the bacteria from binding to the tooth surface.[34] Akande et al (2004) observed a significant reduction in microbial count after use with CPC.[35] A similar finding was found with Okuda et al. (1998) where there was 65% reduction of microbial adherence after a rinse with CPC.[36] This study agrees with the above mentioned study in that, even though not as effective as CHX, CPC also provided microbial inhibition on to the acrylic plate surfaces. But when compared to CHX the Streptooccus mutans colonies were not effectively removed from the plate surface. Lessa et al. found out that 52.9% of the plates disinfected with CPC showed score 1, 11.7% showed score 2, and 35.2% showed score 3.[1] In this study, better results followed, when the plates were disinfected with CPC, 6.6% of the plates showed score 0, whereas 60% of the plates showed score 1, and 33.3% showed score 2. The comparison of both the sprays other than the control, showed a better result with CHX, in both the studies. In all cases, spraying with CHX yielded a greater reduction of in Streptococcus mutans colonies/biofilm. Its efficacy in decreasing Streptococcus mutans levels in saliva and dental biofilm of orthodontic patients was demonstrated by Amitha and Munshi.[37] Non adoption of disinfection policies at home and in the clinical setting is probably because general dentists and orthodontists are not aware of how contaminated orthodontic appliances might be. Further studies comparing a wide range of antimicrobials for the disinfection of removable orthodontic appliances has to be carried out that can help in incorporating ideal protocols for disinfection of acrylic plates.

VI. Conclusion

With the results of this study, it can be concluded that 0.12% CHX sprays on the acrylic surfaces of removable orthodontic appliances is an effective method of disinfecting them, compared to 0.05% CPC.

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