Evaluation of the anti-gingivitis effect of a chlorhexidine (CHX) mouthwash with or without an Anti-Discoloration System (ADS) compared to placebo during experimental gingivitis

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**Background:** Chlorhexidine is the most effective antiplaque agent to date. However, the side effects like teeth discoloration and bad tastes are its drawbacks. A new product in the market was proposed to minimize these side effects while maintaining the antiseptic effects of chlorhexidine. It’s a 0.12% chlorhexidine with anti-discoloration system (ADS). The aim of this clinical trial based on experimental gingivitis model was to evaluate the effectiveness of the chlorhexidine with ADS on preventing stains, plaque accumulation and gingivitis.

**Material and Methods:** This double blinded, parallel, randomized and controlled clinical trial (RCCT) was conducted on 26 dental students (aged 18–20 years) with healthy periodontium. After a preparatory period (3 weeks) of professional prophylaxis and optimal tooth brushing, the participants were randomly assigned to three groups: group P (Placebo), group T1 (0.12% Chlorhexidine without ADS) and group T2 (0.12% chlorhexidine with ADS). During the 3 weeks of experimental period, the mechanical oral hygiene practice was paused. The participants were asked to rinse with 10 ml of mouthwash sample or placebo twice daily. Both participants and examiners were blinded by the group allocation. The clinical parameters, including: discoloration index (DI), plaque index (PII) and gingival index (GI), were assessed by the same examiners on day 0, 7, 14 and 21.

**Results:** All 26 participants completed the study with good compliance. On baseline there was no significant difference on any parameters between any of the groups. Throughout the study, there was no significant difference on the mean DI, PII or GI between group T2 and group P. On the contrary, there was significant difference on the mean DI between group T2 and group T1 on day 14 (0.19±0.15 vs. 0.87±0.56, p=0.007) and on day 21 (0.21±0.17 vs. 1.13±0.59, p=0.001). Significant difference was also found on the mean PII between group T2 and group T1 on day 7 (0.89±0.40 vs. 0.13±0.09, p<0.001), day 14 (1.32±0.46 vs. 0.25±0.12, p<0.001) and on day 21 (1.45±0.39 vs. 0.25±0.19, p<0.001). Similarly, there was also significant difference found on the mean GI between group T2 and group T1 on day 14 (0.71±0.39 vs. 0.14±0.10, p=0.002) and on day 21 (1.12±0.41 vs. 0.18±0.19, p<0.001).

**Conclusions:** The chlorhexidine with ADS appeared to be effective in preventing stain on the teeth. However, the ability of chlorhexidine mouthwash of preventing plaque accumulation and gingivitis was also greatly hampered by the addition of ADS. In fact, the chlorhexidine mouthwash with ADS showed no superior effect over water on maintenance of oral hygiene or prevention of gingivitis.
However, two pronounced side effects – superficial staining of the teeth and altered taste perception – were recognized almost immediately.\(^5\) That may compromise patient compliance for esthetic reasons. The mechanisms of chlorhexidine staining have been proposed.\(^6-8\) They included that the degradation of the chlorhexidine molecule to release parachloroaniline, catalysis of Maillard reactions, protein denaturation with metal sulfide formation and precipitation of anionic dietary chromogens.

Hefti and Huber (1987) studied the effect on early plaque formation, gingivitis and salivary bacterial counts of mouthwashes containing hexetidine/zinc (HZA), aminofluoride/tin (ASF) or chlorhexidine (CHX).\(^9\) They found HZA and CHX almost completely inhibited plaque accumulation and gingivitis. ASF was less effective but still reduced plaque significantly compared to the neutral control. It is interesting that yellow-brown tooth staining was common with all active rinsing solutions in this study. This side-effect is well-known and may be attributed to the cationic nature of the antisepsis.\(^7\) Therefore, evidences seem to imply that staining is an inevitable effect if antiseptic mouthwash actually works.

Hofer and Meier (2011) assessed the biofilm reduction and discoloration potential of a new 0.05% chlorhexidine (CHX) digluconate solution, containing additional essential oil and alcohol components.\(^10\) The results showed that the test solution exhibited an oration potential of a new 0.05% chlorhexidine (CHX) digluconate solution, containing additional essential oil and alcohol components.\(^10\) The results showed that the test solution exhibited an antimicrobial activity but the composition seemed to hamper its effectiveness, although by the trend less staining. Recently, a chlorhexidine product with anti-discoloration system (ADS) was launched in the market. A comparative study was done to confirm the effectiveness of 0.2% chlorhexidine with ADS.\(^11\) The study was single-blinded. In the experimental period, volunteers maintained their usual oral hygiene habits, besides, they rinsed twice daily with mouthwash (0.2% chlorhexidine) with or without ADS. Obviously, subject’s home oral hygiene may become a major confounder of plaque accumulation. Although the effect of ADS is statistically significant, the ability of this new product to prevent plaque accumulation and gingivitis is highly questionable.

Cortellini, et al. (2008) evaluated the side effects, the staining in particular, the patient acceptance, and the efficacy of a 0.2% CHX mouthwash containing ADS compared with a 0.2% CHX alone, after periodontal flap surgery.\(^12\) This clinical trial was carried out on 48 consecutive patients, lasting for 2 weeks. After periodontal therapy, the patients were prescribed to rinse twice daily for 1 week with test or control CHX. No brushing or inter-dental cleaning of surgical area was allowed. At week 1, after suture removal, patients received full-mouth prophylaxis and were given a second bottle of mouthwash, reversing the products. At week 1 and 2, staining, gingival parameters at the surgically treated sites and patient perception and acceptance of two mouthwashes were recorded. The gingival variables included gingival inflammation, tissue inflammation detected around the surges, gingival swelling and presence of granulation tissue at experimental sites, all using same scale (0–absent, 1–present). The results showed that CHX ADS caused less pigmentation and was as effective as CHX without ADS in reducing gingival signs of inflammation in the post-surgical early healing phase. They concluded that the use of CHX ADS could be of value in treatment protocols in which the patient compliance with a CHX mouthwash prescription is relevant.

Later, Guggenheim & Meier (2011) compared the antimicrobial effects of chlorhexidine mouth rinses available on the Swiss market by the Zurich pliospecies biofilm model.\(^11\) CHX (0.2% and 0.12%) with ADS was included. They found that all solutions containing CHX reduced the number of microorganisms in biofilms. CHX with or without ADS fell into different groups according to their efficacy. The two CHX with ADS solutions reduced the number of total CFU by 3 log 10 steps. This seems sufficient for a long-lasting prophylactic application. The CHX control reduced the number of total CFU by 7 log 10 steps. These mouthrinses are predestined for short-term therapeutic use. However, reversible side effects must be taken into account. It has thus far not been possible to formulate CHX products with effective ADS (Anti Discoloration System) additives without reducing antimicrobial activity.

Hence, controversy exists about the clinical efficacy of the chlorhexidine products with ADS. Consequently, a clinical validation of such products appears necessary. In the present study, the 21-day experimental gingivitis model was used. This model is an established noninvasive model in humans for investigating the induction and resolution of inflammation in response to increasing bacterial accumulation.\(^14\) The design enables a study to be performed over 35 days in a well controlled manner. So far it is the most accurate clinical study model to access how medication or compounds in dentifrices affect plaque accumulation and gingival inflammation. The aim of this RCCT is to test the null hypothesis of no significant differences in clinical parameters assessed during a full-mouth experimental gingivitis period of 3 weeks in subjects who use a 0.12% chlorhexidine (CHX) mouth-rinse either with or without an ADS (Anti Discoloration System) compared to age/gender matched controls rinsing with a placebo (water with flavor additive).

**MATERIAL AND METHODS**

This study was a single-centre, double blinded, parallel, randomized and controlled clinical trial (RCCT) that was conducted on 26 dental students (aged 18-20 years) with healthy periodontium. The participants were recruited from the Prince Philip Dental Hospital (Dental Faculty of the University of Hong Kong) through advertisement and well informed about the purposes, risks and benefits of the study. Signed consent forms were acquired before beginning of the study. The study was approved by Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 11-417). The inclusion criteria for screening the participants were: the participant should be a non-smoker; systemically healthy; at least having 24 teeth in the functional dentition, excluding third molars; having no active caries; not using systemic antibiotics within 3 months prior to enrolment; clinical diagnosis of periodontal health or gingivitis as determined by the presence of pocket probing depths (PPD) not exceeding 4 mm with concomitant bleeding on probing (BOP).

Before commencement of the study, the participants were screened according to the inclusion criteria. After a preparatory phase of prophylaxis and 3 weeks of optimal oral hygiene practice, the plaque and gingivitis scores of the participants approached zero. The participants were then asked to abolish all measures for mechanical plaque control for a period of 3 weeks according to the experimental gingivitis model.\(^14\) During this period, the participants were randomly assigned to one of these three groups:

**Group P** twice daily rinse with 10 ml of a placebo (pure water with flavored additive) solution

**Group T1** twice daily rinse with 10 ml of 0.12% chlorhexidine solution

**Group T2** twice daily rinse with 10 ml of 0.12% chlorhexidine with ADS (Anti Discoloration System) solution (Curasept ADS 212)

During the experimental period, the participants were asked to rinse with the mouthrinse sample or the placebo for 60 seconds in the dental office twice a day. The samples were distributed to the participants by a dental assistant who was unaware of the purpose of the study. The containers for distribution were unlabelled, which means that both the participants and the examiners were blinded of the group allocation.

At day 0, 7, 14 and 21 of the experiment, clinical examinations were performed from central incisors to the second molars in each participant. Discoloration index (DI) was recorded at three aspects on each tooth (mesial, buccal and distal). The criteria for the discoloration index (DI) are indicated in Table 1.\(^15\) Plaque and gingivitis indices were assessed at 4 aspects of each tooth (mesial, buccal, distal and lingual) using the criteria of the Plaque Index System (PII) (Sillness & Loes 1964) and the Gingival Index System (GII) (Loe & Sil-
ness 1963). At each visit, the parameters were assessed by the same examiner, who was marked by the allocation of the test and control to avoid examiner bias and calibration bias. Upon completion of the experimental period, mechanical daily plaque control measures were reinstated and the participants were re-examined after 2 weeks to ensure their periodontal health. Following the experimental period, another professional cleansing was performed to remove plaque and possible stain on the teeth.

Statistical analysis

In the experimental gingivitis model, an n=8 provided a power of 80% to determine a difference in mean Gingival Index values of 0.2 between groups with an α-error of 0.05 and a β-error of 20%, given standard deviations of 0.1 derived from previous experimental gingivitis studies. All statistical analyses were conducted using SPSS v. 19 (spss inc. Chicago, il US). The outcome variables were analyzed using independent t-tests and paired t-tests. Descriptive statistics and frequency analyses were performed. Between groups comparisons were performed applying independent t-tests. Paired t-tests were used to study over time differences within each group for the clinical parameters.

RESULTS

All 26 participants followed the rinsing protocol strictly, after 3 weeks of experimental period, no one dropped out from the study. After prophylaxis and three weeks of optimal plaque control (baseline), the clinical parameters showed no significant difference between the groups: the mean DI≤0.03, mean GI≤0.06, and mean PII≤0.06.

Table 2 presents that during the experimental period, the mean DI in Group P remained the same. On the contrary, compared with baseline, the mean DI in Group T1 increased significantly on Day 14 (p<0.002) and Day 21 (p<0.001). And there was a constant significant increase between Day 14 and Day 7 (p=0.002) and between Day 21 and Day 14 (p=0.049). The mean DI in Group T2 also had a significant increase since Day 14 (p=0.012), although this increase was minor, and there was no further increase between Day 21 and Day 14. Throughout the study period, there was no significant difference of DI between Group P and Group T2 at any of the time intervals. On the other hand, the mean DI between Group T2 and Group T1 were significantly different from each other on Day 14 (0.19±0.15 vs. 0.87±0.56, p=0.007) and on Day 21 (0.21±0.17 vs. 1.13±0.59, p=0.001). Fig 1 demonstrated that the mean DI in Group P did not change over time. In contrast, the mean DI in Group T1 increased dramatically since day 14. This is a correct finding since the traditional Chlorhexidine does stain the teeth. The chlorhexidine mouthwash with ADS is claimed to have no side effect of staining the teeth, overall this was a true finding as there was no significant difference on mean DI between this new product and placebo. However, there was still a slight increase of the mean DI in Group T2 (p=0.012), although this increase is minor compared with group T1.

In table 3, the mean PII in both Group P and Group T2 increased considerably from Baseline to Day 14, although there was no further significant increase from Day 14 to Day 21 in both groups. Consequently, there was no significant difference of PII between the two groups at any of the time intervals, meaning the chlorhexidine mouthwash with ADS failed to prevent plaque accumulation throughout the experimental period. The mean PII was also significantly increased from Baseline to Day 14 in group T1, but the increase is much less compared with the other two groups, in fact, there was a constant significant difference between Group T1 and Group T2 at all time intervals (0.13±0.09 vs. 0.89±0.40 on day 7; 0.25±0.12 vs. 1.32±0.46 on day 14, and 0.25±0.19 vs. 1.45±0.39 on day 21, p<0.001 for all three time intervals). Figure 2 shows that the mean PII in Group P and Group T2 increased greatly and this increase was constant over time. On the contrary, the mean PII in Group T1 stayed at a low level over the whole study period. Corresponding to the mean PII, Table 4 shows that in both Group P and Group T2, there was a continuous significant increase of mean GI over time, and there was no significant difference between the two groups at any of the time intervals. This implies that the chlorhexidine mouthwash with ADS didn’t show any positive effect on preventing gingivitis either. In Group T1, the mean GI increased slightly on Day 14 (p=0.002), but this increase didn’t progress further. Between Group T1 and Group T2, significant difference of mean GI was found on Day 14 (0.14±0.10 vs. 0.71±0.39, p=0.002), and Day 21 (0.18±0.19 vs. 1.12±0.41, p<0.001). Figure 3 also de-
Figure 4. Frequency distribution of Discoloration Index (DI) in the three groups on Day 7 (a), Day 14 (b), and Day 21 (c)

Figure 5. Frequency distribution of Plaque Index (PII) in the three groups on Day 7 (a), Day 14 (b), and Day 21 (c)

Figure 6. Frequency distribution of Gingival Index (GI) in the three groups on Day 7 (a), Day 14 (b), and Day 21 (c)

picts this trend, while the mean GI in Group P and Group T2 both soared up in the same pattern, the mean GI in Group T1 stayed at a low level.

Figure 4 depicts the frequency distribution of discoloration index (DI) in three groups over time. On day 7, there was no apparent difference between the three groups. From day 14, Group T1 showed less than 40% of DI=0 sites while in the other two groups the DI=0 sites stayed above 80%. On day 21, there were only about 20% sites presented with DI=0 in Group T1. While in the other two groups the frequency distribution of the GI scores stayed the same as day 14. Statistically, there was no significant difference of sites percentage of DI=1 between Group P and Group T2 throughout the study. However, on day 14, there was significant difference of sites percentage of DI=2 between Group P and Group T2 (p=0.039), meaning the stain on teeth in Group T2 was a little worse than Group P on day 14. There were constant significant differences of sites percentage of DI=0, DI=1 and DI=2 between Group T2 and Group T1 on day 14 and day 21, which means the chlorhexidine mouthwash with ADS does prevent stain on teeth when compared with traditional chlorhexidine mouthwash.

Figure 5 demonstrates the frequency distribution of Plaque Index (PII) in three groups over time. It was an obvious finding that since day 7, large amount of plaque had already accumulated on most of the sites in Group P and Group T2, the percentage of sites presented with PII=1 and PII=2 almost reached 80% in both groups. In contrast, there were only less than 20% sites presented with PII=1 and PII=2 in Group T1. This difference between groups continued on day 14 and day 21. On day 21, there were more than 60% sites in Group T2 and almost 80% sites in Group P presented with PII=2. Actually, there was no significant difference of percentage of sites presented with PII=0, PII=1 and PII=2 between Group P and Group T2 on day 21. However, there was constant significant differences of percentage of sites presented with PII=0, PII=1 and PII=2 between Group T1 and Group T2 on day 21 (p<0.001). This result further proves that the Chlorhexidine mouthwash with anti-discoloration system did not prevent plaque accumulation.
In figure 6, the frequency distribution of Gingival Index (GI) in three groups over time illustrates the same trends. Since it takes time for gingival tissue to respond with plaque accumulation, the difference between groups happened from day 14 instead of day 7. On day 14, there were almost 60% sites presented with GI=1 and GI=2 in both Group P and Group T2, while in Group T1 there was only less than 20% sites presented with GI=1 and no site with GI=2. On day 21, there were almost 80% sites presented with GI=2 in Group P and 50% in Group T2, the sites with GI=0 were less than 20% in both groups. On the other hand, the sites presented with GI=0 stayed around 80% in Group T1 on day 21. Statistically, there was no any significant difference of percentage of sites presented with GI=0, GI=1 and GI=2 between Group P and Group T2 at any time intervals. And there were constant significant differences of percentage of sites presented with GI=0, GI=1 and GI=2 between Group T1 and Group T2. Again, the results showed that the Chlorhexidine mouthwash with anti-discoloration system did not prevent gingivitis during the experimental period.

**DISCUSSION**

All the participants rinsing with a placebo solution revealed increased mean PI, which after 3 weeks led to the development of generalized gingivitis. These results are in agreement with the model of experimental gingivitis. The present study also confirmed that the daily rinses of a 0.12% solution of chlorhexidine resulted in buccal gingival tissue clinically resembling those achieved with generally accepted mechanical oral hygiene practices.

Since our focus is the staining side effect of chlorhexidine, the initial question is – whether ADS works or not. The results from the study showed that the mean DI in this group of participants rinsing with the chlorhexidine mouthwash with ADS still had a significant increase between day 7 and day 14. However, overall there was no difference in terms of mean DI between the chlorhexidine mouthwash with ADS and placebo. So the answer to the first question is: the chlorhexidine with ADS does eliminate the side effect of staining. It was also noticed that 0.12% chlorhexidine 10ml twice daily mouth rinse yield a low DI score during the first week. This may be explained by the advantage of the low concentration as 0.12% compared with 0.2% chlorhexidine mouth rinse, while the two solutions offer the same clinical benefits when used at appropriate similar doses.

Another important question is: does ADS comprise the anti-plaque effect of chlorhexidine? The result showed that the chlorhexidine with ADS did not prevent plaque accumulation at any of the time intervals. The amount of plaque accumulated through the experimental period was not significantly different between the chlorhexidine with ADS group and the placebo group. Moreover, the amount of plaque accumulation from the chlorhexidine with ADS group was always significantly more than the positive control – the chlorhexidine without ADS. As a consequence to the plaque accumulation, the mean GI was also constantly rising in the chlorhexidine with ADS group, and there was again no statistical difference between these two types of chlorhexidine on day 14 and day 21. This is in agreement with a recent in vitro study. In that study, based on the comparison of colony forming unit (CFU) from discs covered with biofilms and exposed to CHX with or without ADS, it was shown that the addition of ADS reduced the antimicrobial ability of chlorhexidine. Similarly, Hofer et al. (2011) has assessed the biofilm reduction and discolouration potential of a new 0.05% chlorhexidine (CHX) digluconate solution, containing additional essential oil and alcohol components, compared with that of standard control CHX solutions (0.05% and 0.2% CHX). They found that the composition seemed to hamper its effectiveness and it is statistically significant, although by trend less staining on restorative materials.

Since yellow-brown tooth staining was common with all active rinsing solutions, this side effect of chlorhexidine is attributed to its cationic nature. Chlorhexidine binds strongly to bacterial cell membranes. At low concentration this causes increased permeability with leakage of intracellular components. At high concentration, chlorhexidine causes precipitation of bacterial cytoplasm and cell. In the mouth chlorhexidine readily adsorbs to surfaces including pellicle-coated teeth. A review suggested that plaque inhibition was derived only from the chlorhexidine adsorbed to the tooth surface. It is possible that the molecule attaches to pellicle by one cation leaving the other free to interact with bacteria attempting to colonize the tooth surface. This mechanism would, therefore, be similar to that associated with tooth staining. Chlorhexidine is a highly active, so it can be easily deactivated by any anionic compound, including the anionic surfactants commonly used as detergents in toothpastes and mouthwashes, anionic thickeners. Accordingly, the anti-stain effect of ADS may be from its deactivation.
of chlorhexidine, which can explain the compromised anti-plaque effectiveness of the test group in the present study. The inability of the chlorhexidine with ADS to prevent plaque accumulation as resulted from the present study conflicts with some other clinical trials. All these three studies did not find any statistical difference of the effect on preventing plaque accumulation and gingivitis between the chlorhexidine with or without ADS. To date, actually the present study is the only clinical trial that revealed the opposite results. It’s interesting to notice that the present study was also the only study that adopted the study design of experimental gingivitis. The experimental gingivitis model has been a gold standard for testing the efficacy of dental hygiene products. The principle of the experimental gingivitis is that, in periodontally healthy participants, through three weeks of refraining from mechanical plaque control, starting from a state of gingival health and no clinically detectable plaque, gingivitis should be developed in 10–21 days. Therefore, if a dental hygienic product is applied during this period, its efficacy in preventing plaque and gingivitis will be established without bias or confounding factors. Results generated from this type of design are usually most compelling. However, the above mentioned three clinical trials had different approaches. The first study by Bernardi, et al. (2004) maintained the routine brushing of the participants during the whole study period, so it was hard to say whether the results were due to the effect of the mouthwashes or the brushing. The second study by Cortellini, et al. (2008) specifically tested the effect of this product on maintaining oral hygiene after flap surgeries. The test mouthwash and the positive control (CHX without ADS) were only applied for one week in the patients. It is therefore questionable that there can be any advanced plaque accumulation or development of gingivitis in such a short period. Moreover, in that study, instead of using GI and PII as parameters, the gingivitis was only recorded as positive or negative, which could be very subjective. The third study by Solis, et al. (2011) tested the product in patients with chronic periodontitis instead of periodontally healthy participants, and they neglected to report baseline examinations for the gingival status. In addition, this study also maintained other mechanical oral hygiene practice (brushing, inter dental brushing and flossing) during the experimental period. The study design of the present study strictly followed the principles of experimental gingivitis. Furthermore, objective clinical indices – plaque index system (PlI) (Silness & Loe 1964), gingival index system (GI) (Loe & Silness 1963) and discoloration index (DI) (Lang & Raber 1981) – were adopted to assess clinical outcome with clearly preset criteria. Therefore, the results generated from the present study are more justified compared with the other three clinical trials. In conclusion, the chlorhexidine with ADS appeared to be effective in preventing stain on the teeth. However, the ability of chlorhexidine mouthwash of preventing plaque accumulation and gingivitis was also greatly hampered by the addition of ADS. In fact, the chlorhexidine mouthwash with ADS showed no superior effect over water on maintenance of oral hygiene or prevention of gingivitis.

ACKNOWLEDGEMENT

The authors are grateful to the Periodontal Department of the Prince Philip Dental Hospital for their kind assistance in preparatory treatment. The authors would also like to thank the dental assistants in the Department of Comprehensive Dental Care. The authors report no conflicts of interest related to this study.
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