A Comparison of the Efficacy of Non-alcoholic 0.05% Cetylpyridinium Mouthwash and Chlorhexidine Alcohol-free Mouth Rinse on Patients with Gingivitis: A Double-blind Randomized Controlled Trial

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Abstract
Background: Oral health preservation is of major importance in patients undergoing periodontal surgery. Tooth brushing is a competency that is challenging to perform. Therefore, materials with biochemical properties that can control the tooth plaque biofilm are used.
Objective: The purpose of the study was to determine the clinical outcome of alcohol-free Chlorhexidine (AFCHX) 0.12% oral rinse in comparison with 0.05% Cetylpyridinium chloride (CPC) non-alcoholic mouthwash in outpatients with periodontitis.
Methods: Patients with periodontal diseases attending the Department of Periodontology, Dental School of the Islamic Azad University of Medical Sciences, Tehran, Iran received a double-blind randomized controlled trial. In this study, a total of 38 patients with gingivitis and pocket depth ≤4 mm were randomly allocated into two equal study groups and followed up within 90 days: Group A (performed mouth rising twice a day with the liquid including 10 ml of the 0.05% CPC without ethanol), Group B (performed mouth rising twice daily with 10 ml of the alcohol-free 0.012% chlorhexidine solution). Furthermore, periodontal parameters and Stain Index (SI) were obtained at baseline and for both groups one month later. ANOVA was used to analyze the data. Statistical significance was defined as p-value <0.05.
Results: Full-mouth plaque index (PI) was non-significantly different in the two groups, and the decline was 35% for the CPC group and 44% for the individual AFCHX group. Moreover, reductions in BOP were statistically significant for the CHX group after the 12th week (p=0.0001). Additionally, the PD drop within 30 days was statistically significant (1.92±0.30) AFCHX group and (2.30±0.36) CPC group (p=0.002). Meanwhile, CPC and AFCHX plan of teeth were also compared, and greater rates of staining were observed in AFCHX areas of teeth at days 7 to four weeks.
Conclusion: The findings indicated that the application of a therapeutic CPC mouth rinse, twice daily aids control plaque as well as gingivitis, and periodontitis surpassing traditional plaque control, although not as much as AFCHX mouthwash.
Trial registration: The study was registered under Identifier No. NCT02756377 at ClinicalTrials.gov.
Funding: This study was entirely sponsored by researchers and did not receive a specific grant from any funding agency.
Keywords: Cetylpyridinium, Chlorhexidine, Periodontal Disease
Note: This study has followed the CONSORT Statement, which is an evidence-based, minimum set of recommendations for reporting randomized trials. It offers a standard way for authors to prepare reports of trial findings, facilitating their complete and transparent reporting, and aiding their critical appraisal and interpretation.

Abbreviations / Acronyms:
AFCHX: Alcohol-free Chlorhexidine; CHX: Chlorhexidine; CPC: Cetylpyridinium chloride

1. Introduction
Dental plaque plays a major role in the progress of gingivitis, which in turn can develop into periodontitis (1). Periodontal diseases occur when pathogenic microbial tooth plaque acts on a suspected host (2). Additionally, traditional removal of bacterial dental plaque is the most effective technique to control such conditions as tooth caries, gingivitis, and for the prevention of rising periodontal disease (3, 4). However, daily brushing has been reported to eliminate plaque deposits only partially (5); the large preponderance of gingivitis means that the conventional self-performing plaque is inadequate; the resistor restricts gingival inflammation (6). Subjects’ attempts, nevertheless, are frequently jeopardized by the appearance of hard-to-reach regions as well as an insufficient proficiency, little stimulus, and lack of admission. As a result, the application of antimicrobial mouthwash in conjunction with the usual oral health regimens has been a factor for intensifying plaque removal (7, 8). The adjunct usage of anti-microbial factors can benefit the adjunct control of supra-gingival biofilm. Many anti-microbials have been examined as adjuncts to conventional tooth plaque biofilm control to improve the results achieved in oral home-care (6). To this end, there is a type of mouthwash, of which Chlorhexidine (CHX) has been presented as the routine efficient chemical factor to regulate dental plaque (9-11). The high potency of CHX, a cationic bisbiguanide molecule, has been ascribed to its high substantively in the mouth, as well as its bactericidal and bacteriostatic operations (12). In addition, CHX is regarded as the gold standard material in regulating oral biofilm development in the mouth or microbial pathogens change by mouth-aerosols (13-18), which is a reason for its widespread antibacterial spectrum (17, 19). As an adjunct to conservative oral hygiene systems, the benefit of antimicrobial mouth rinses is regarded a complement to improve plaque control (20-23). Particularly, CPC, which carries prolonged records of safe and proficient intra-oral utilization has commonly been applied as an antibacterial component to improve clinical efficacy (24, 25). CPC, which is a cationic quaternary ammonium mixture, has been offered as an option to CHX. CPC is a useful yield for regulative dental plaque biofilm development. It declines the bacterial plaque on the tooth surface that produces periodontitis, and thus inhibits the inflammation of the gums, as well as bad breath. Although it is marginally more effective than CHX at reducing dental plaque biofilm formation, it is distinguished by the appearance of significantly more uncommon adverse effects, such as tooth staining or color change. It is caused by CHX mouthwash. The anti-plaque effect of CPC can be attributed to the cationic component of CPC, which simply binds to the negatively charged proteins of the oral tissue (26-28). Quirynen et al., on the other hand, described the possibility of a unique 0.12% CHX +0.05% CPC non-alcoholic formulation as an effective anti-dental plaque cause for long-range control with diminished individuals' side effects (29). The present study evaluated the anti-plaque impacts of the 0.05% CPC non-alcoholic formulation as an effective anti-plaque compared to 0.12% CHX. In both groups, periodontal parameters and extrinsic tooth staining were assessed.

2. Material and Methods
2.1. Research design and contributors
This double-blind randomized clinical trial was conducted from December 2014 to May 2015. It was implemented on 38 subjects admitted to the Department of Periodontology, Dental Faculty of the Islamic Azad University of Medical Sciences, Tehran, Iran.

2.2. Selection Criteria
The inclusion criteria were as follows:
1) Patients with gingivitis;
2) Age >18 years old;
3) Having at least 20 teeth

The exclusion criteria were:
1) Patients with a history of systemic illness;
2) Patients with no orthodontic devices or removable prostheses (to avoid food particles and plaque that can accumulate on the appliance, this will help prevent plaque build-up);
3) Patients with a known allergy to any of the components of mouthwashes;
4) Patients who had taken antibiotics or anti-inflammatory medication for the last 3 months;
5) Patients with cavitated caries lesions;
6) Pregnant or breastfeeding women;
7) Smokers;
8) Patients under periodontal therapy through for the last six months.

2.3. Intervention and measurements
On the first visit, all participants received scaling and root planning, as well as proficient prevention and inhibition; oral hygiene instructions, including double-daily brushing, were delivered. The contributors cleaned their teeth for at least two minutes using the Bass brushing method, and these patients were randomly and evenly divided into two groups: Group A: rinsed their mouths twice a day with a suspension containing 10 ml of 0.05 CPC (VI-one, Rojin, Cosmetic-Lab, Kurdistan-Iran), Group B: performed twice daily mouth-rinse with 10 ml of the 0.012% AFCHX (Kim Laboratories, Spain). Patients in both groups were assigned a container at random to rinse the objects with mouthwash for 60 seconds once in ante meridian and at nighttime following tooth cleaning or brushing their teeth. It was advised to the subjects to abstain from eating or drinking for half an hour after using the mouthwash.

2.4. Outcomes
Split-mouth is justified, and the study results are more perfect. Intersecting tests for therapeutic procedures or rapidly changing conditions may be unfeasible and immoral. A parallel-team and a split-mouth randomized, double-blind, controlled trial were used in the study, and all periodontal parameters were gathered by a blinded experiment. Following that, weekly follow-ups were performed for four weeks. Individuals returned every week for a four-week interval study. Plaque index (Plaque index was scored according to Turesky’s (30) and O'Leary's index) (31) and probing depth (PD) were placed with slight force probing into the gingival crevice/sulcus by a periodontal probe. Within 30 seconds of probing, the bleeding index (BI) was recorded as present or absent at six sites per tooth (Score 0: No bleeding following probing. Score 1: A single separate bleeding point visible after probing) scores (32, 33), and Stain index (SI using scores on the Lobene Stain Index; scored 0-3. Where 0 = no stain, 1 = light stain, 2 = moderate stain and 3 = heavy stain) (34).

2.5. Sample size
According to prior research, the sample size was calculated by detecting differences in the primary result of periodontal disease measurements probing pocket depth (PD) from the first day to the last day of follow-up. The sample size for the study was determined using the MINITAB's “two-sample t-test sample size calculation” tab, with α=0.05, β=0.05 mean a deviation of equal to 1.65 and merged standard variation=1.4. Therefore, each group required at least 17 cases.

2.6. Randomization and Blinding
A single investigator prepared the randomization, who had no role in the treatment of contributors. Qualified participants who affirmed their continuing dedication to the investigation were block-randomized for qualified participants who confirmed their continued commitment to the experiment were block-randomized for operation in a double-blind manner; block randomization with a 4:1 allocation was used. Total mouthwashes were fitted in the same glass bottles, which were labeled with (A, B). Each participant selected one symbol from a box, which determined his/her subsequence of the mouthwash use. The order of mouthwash use was randomized, and the entire clinical research was supervised by a calibrated inspector; also, prior to opening the trial, one examiner (a periodontist) was trained to the calibration manner twice to minimize the examiner's reliability. The examiners had been calibrated for one week prior to the start of the experiments, and the periodontal assessment was carried out by a single calibrated examiner. An investigator evaluated the subjects twice, once at 0 and once for two days. The calibration was confirmed because 90% of the studies were recorded within the 1.0 mm range.

2.7. Statistical analysis
Data were analyzed by IBM© SPSS© Statistics 21 (IBM© Corp., Armonk, NY, USA). ANCOVA test was used to control potential distortion variables. Furthermore, a t-test was used for the paired sample's within-group association, while the judgment of variation (ANOVA) was used for the within-group comparison, using the average variance valences of the PI, BI, and PD on the first day and one month later. A p-value of <0.05 was determined to be statistically significant.
2.8. Ethics of research
Inquiry Ethics Permission and Consent were obtained from the Ethical Commission of Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran (Ref: 24623). The study was registered under Identifier No. NCT02756377 at ClinicalTrials.gov. All participants were informed both orally and in writing. The contributors provided their consent to participate in the study; also, we were required to keep all of the participants' information private. Oral rinse was made available to subjects on a regular basis and was validated by the Ministry of Health and Medical Education, and was available on the market.

3. Results
All 40 cases were tested; none of the cases in either group complained about taste when rising; nevertheless, during the examination, two participants who seemed younger complained about the burning sensation, as well as piercing pain after rinsing with AFCHX. Moreover, Two CPC group participants withdrew from the treatment for reasons unrelated to medication use. Figure 1 depicts the experimental protocol. There were no significant differences in clinical periodontal scores between the CPC and AFCHX groups at the start of the study. In terms of the clinical periodontal index, after 7 days, the outgrowth of periodontal therapy showed a statistically significant decrease in PI, BI, and PD in both groups (Table 1). There was an increase in full-mouth PI in both A and B groups; however, there was a statistically significant variation in full-mouth PI within the two groups. Aside from that, there was no statistically significant difference in full-mouth PI between the two groups at any meeting. This reduction was 35% for the CPC group and 44% for the chlorhexidine group (Table 1).

The BI sign did not change in the two groups at the first visit, however there was a statistically significant decline in BI rates from baseline to 30 days in both groups. At the final visit or 90th day, the decrease in BI mark was greater in group A (0.150±12) compared to group B (0.190.35) (p=0.0423) (Table 1). After the 12 weeks (p=0.0001), the reduction in BOP was statistically significant for the CHX group. The clinical periodontal parameters revealed that PD did not differ between the groups. There was a statistically significant decrease in PD rates in both groups when compared to the initial visit; While the AFCHX group was compared to each other, the decrease in PD at 90 days was statistically significant (1.92±0.30) CHX group and (2.30±0.36) CPC group (p=0.002) (Table 1). At the first visit, the severity of the stain as measured by the discoloration index did not differ between experimental groups. Meanwhile, the CPC and AFCHX tooth plans were compared, and higher rates of staining were seen in AFCHX areas of the teeth at days 7 to 12 weeks (Table 1).

![ConsorT 2010 Diagram of the study](image-url)

**Figure 1.** CONSORT 2010 Diagram of the study
Table 1. Full-mouth PI* values and BI* values for all groups at different mouthwash and time; and Change in pocket depth across time and satin index in the study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Baseline</th>
<th>One month</th>
<th>Three months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index (Tursky)</td>
<td>CHX</td>
<td>2.77±0.674</td>
<td>1.58±0.689</td>
<td>0.8±0.447</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
<td>2.3±1.03</td>
<td>1.49±0.617</td>
<td>0.98±0.497</td>
<td></td>
</tr>
<tr>
<td>Plaque index (O'Leary)</td>
<td>CHX</td>
<td>69.70±17.29</td>
<td>42.76±9.93</td>
<td>29.06±7.25</td>
<td>0.2205</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
<td>73.88±1.66</td>
<td>46.23±9.15</td>
<td>32.25±7.63</td>
<td></td>
</tr>
<tr>
<td>Bleeding Index</td>
<td>CHX</td>
<td>0.50±0.403</td>
<td>0.29±0.488</td>
<td>0.15±0.350</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
<td>0.43±0.5</td>
<td>0.26±0.51</td>
<td>0.15±0.258</td>
<td></td>
</tr>
<tr>
<td>Pocket depth</td>
<td>CHX</td>
<td>2.9±0.820</td>
<td>2.3±0.54</td>
<td>1.9±0.301</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
<td>2.8±0.719</td>
<td>2.4±0.50</td>
<td>2.1±0.352</td>
<td></td>
</tr>
<tr>
<td>Stain Index</td>
<td>CHX</td>
<td>-</td>
<td>0.47±0.514</td>
<td>2.54±0.52</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>CPC</td>
<td>-</td>
<td>0.62±0.50</td>
<td>1.56±0.63</td>
<td></td>
</tr>
</tbody>
</table>

* Plaque index (Tursky and O'Leary); ** Bleeding Index (using the Carter and Barnes Bleeding Index)

4. Discussion

The study's main goal was to identify the benefit of the alcohol-free CHX with CPC mouthwash in reducing tooth plaques, alter periodontal tissue healthy, and assess the adverse effects. Loss of hygienic way and inability to attain proper dental brushing can render conventional tooth plaque control ineffective (35). As a result, the usage of mouthwashes may be beneficial in improving plaque biofilm regulation as well as gingiva health by eliminating dental plaque from difficult-to-reach areas. Because of its long-term efficiency in reducing dental plaque, CHX has been recognized as the gold standard among mouthwashes (36). However, it has the problems of creating tooth staining, accumulating alcohol, and altering flavor sensations. These conditions restrict its use primarily to the short term (37, 38). Throughout the years, researchers have discussed the lack of side effects of alcohol in mouthwashes for regular use, and some types of research have indicated the intersection between alcohol use in mouthwashes and oral cancer (39). The study focused on two systems of AFCHX and CPC as an adjunctive method to mechanical plaque inhibition. It was explained that there is a significant difference between the AFCHX and CPC for 30 days on PI, BI, and PD indicators and staining symbols. Furthermore, the results showed that the periodontal parameters improved in all groups. Concerning the CHX group, they were more significant than the CPC group. On the 28th day, we discovered that PI in the AFCHX group was lower than CPC. These findings were consistent with previous studies by Quirynen et al., who claimed that CHX 0.12% and CPC 0.05% can suffice as an oral rinse. The study's findings indicated the possibility of a recent CHX 0.12% CPC 0.05% non-alcohol aggregate as an effective anti-dental plaque including an anti-inflammatory factor with diminished unfavorable individuals adverse influences (12, 29). Brownstein et al. demonstrated that CHX oral-washes, in addition to regular tooth plaque inhibition externally primary limitation, significantly reduced dental plaques, even while the incidence of gingival inflammation persisted permanently after 60 days in participants with moderate-severe gingivitis (40). Corbet found no significant differences in PI in outpatients with gingival inflammation; however, there was a significant difference in gingival BI or bleeding rates for 90 days between the CHX and experimental groups (41). In other studies, Zanatta et al. discovered little anti-tooth plaques and anti-gingivitis impact of CHX mouthwash on earlier tooth plaque shielded areas, as well as the benefit of excluding formed dental biofilm prior to CHX use (42). After four weeks, there were significant differences in gingival inflammation reduction between the CHX and CPC mouthwashes in the study. Earlier research by Türkoğlu showed that using CHX mouth rinse as a supplement to normal plaque prevention could be beneficial in the treatment of plaque-related gingival tissue inflammation (43). Moreover, Becerik indicated CHX could be useful in reducing the subgingival total bacteria count, especially in posterior teeth (44). The data indicated the major obvious effectiveness of CPC mouth rinses on specimens of supra-gingival dental plaque obtained from human cases and established prior clinical examinations manifesting notable impacts of CPC on tooth plaques, including the inflammation of the gingiva (45-48).

CPC is a quaternary ammonium compound that is utilized in a wide range of antiseptic mouth rinse products containing 0.07% alcohol. Daily cleaning and washing with CPC significantly reduce clinical documentation of gingivitis. In certain circumstances, Kiszely et al. (49) used it as a supplement to normal oral health plans. The usage of CPC appeared to result in a significant decrease in tooth plaque rates. According to the PI's estimation, this was for the earlier findings of multiple prior research (49-51). When used in conjunction with normal and routine oral health practices, CPC develops bleeding levels (a sign of gingival health). Reliable results are those that correspond to previous investigations when CPC was utilized (49, 52). Ciancio et al. stated that there was no improvement in
bleeding scores in the test group, which included dental cleaning and brushing. The side effects of quaternary ammonium composites include achromatism, a variety of flavors, and a burning sensation (53). Lobene (1979) studied the effectiveness of CPC on gingivitis and dental plaque for 21 days and found it to be between 1:1000 and 1:2000 (54). The participants cleaned and brushed their teeth as usual, but they also rinsed with 20 ml of CPC for 20 seconds twice a day for 21 days. Witt et al. (2005) (55) investigated the agonist for bacterial and dental plaque qualities of a novel, non-alcoholic, oral-rinsed with CPC. They determined that the CPC mouthwash showed a broad spectrum of anti-microbial processes. Allen et al. discovered that after cleaning and rinsing with 15 ml of a 0.05% CPC mouth rinse for 60 seconds, the CPC group had 28% less plaque biofilm of the tooth, a 63% decrease in tooth plaque accumulation, 24% more moderate gingival inflammation, and 67% less gingival bleeding when compared to the control group (56). In a study conducted by Mankodi et al. (49), patients used 20 ml of a non-alcohol 0.07 percent CPC rinse for 30%, and the CPC group had 16% less plaque and 33% less gingival bleeding than the control group (49). Finally, Stookey et al. evaluated two alcohol-free mouthwashes, one containing 0.075% CPC and the other containing 0.1% CPC (48). Patients used 20 ml in 30 seconds. Drops in plaque and gingivitis were 17% and 23% for the 0.075% CPC group, respectively, and 19% and 20% for the 0.1% CPC group. However, despite differences in items, these investigations revealed that correct CPC compounds have significant anti-plaque and gingivitis impacts (57). Rioboo et al. discovered that the evaluated toothpaste and oral rinse with 0.05% CPC as adjuncts to oral cleanliness and health had insufficient benefits in terms of reducing dental plaque formation and having no effect on gingival inflammation (58). According to Zimmer et al., there was no statistically significant difference among those without alcohol and the alcohol-comprising preliminary washes (59). Achromatism is one of the numerous negative outcomes that have been published. Clinical observations have revealed significant changes in the specific stain trends that follow each cycle of CHX washing and cleaning (60, 61). The present study's findings revealed that there was tooth discoloration in both groups, although there were no statistically significant extrinsic stained teeth during the examination.

During the trial, people in the CHX and CPC groups had no statistically significant extrinsic tooth discoloration, according to Charles et al. (62). Furthermore, CPC oral-rinsed was found to have fewer side effects than CHX mouthwash. Blenman et al. discovered less staining with CPC mouthwash compared to CHX mouthwash, and they stated that patients can use longer-term rinsing time with CPC mouth-rinse because there will be no burning sensation and bitter flavor because it contains no alcohol (63). Quirynen et al., defined the CHX and CPC formalization while comparing it with alternative CHX-alcohol compounds, particularly for the taste and flavor of the yield, but less efficient for the discoloring of the teeth region also tongue (12). The findings of Papaioannou et al. (17) research on discoloring or staining of the tooth cover were incorporated into the current study (61). The present study's findings were consistent with those of Jose et al. on 0.2% CHX mouth rinse including alcohol and non-alcohol mouthwash (65). L. Borrajo et al. on 0.12% CHX, 0.05% sodium fluoride, 11% ethanol, and the similar non-alcoholic mouth rinse (66). According to Charles et al., 0.12% CHX oral rinse significantly increased calculus and the presence of discoloration or stains when compared to EO oral-rinse, and the test group (62). Sreerivasan et al. found that the CPC mouthwash had significantly better antibacterial performance (90% killing), while the CHX mouthwash had the maximum efficiency. Mouth rinses containing 0.05% CPC in an alcohol or alcohol-free base exhibited broad-spectrum of antibacterial effect on supragingival plaque biofilm when compared to a control mouthwash that did not contain CPC (67). Pandit et al. proposed that CPC medications have strong anti-S. Mutans’s dental biofilm capacity (68). Latimer et al. described the use of CPC and sodium fluoride in mouthwash to prevent the bacteria and protect the mouth and dental enamel (69). According to Rosing et al., a mouthwash containing CPC and zinc lactate has significant anti-plaque and anti-gingivitis effects when compared to affirmative and adverse control oral rinses (70). However, the primary constraint of the preliminary examination is a lack of specimen volume. The investigation began with 38 subjects; four of the participants were dismissed for unknown reasons. Microbiological examinations are also required on a large scale and over a long period of time to establish the effectiveness and protection of CPC without alcohol on the health of gingival or periodontal tissues as a comparison to other anti-dental plaque and anti-inflammation agents on the gingiva. Therefore, based on the findings of many studies, including those of the present research, it can be determined which CPC mouth rinses without alcohol can be used as a substitute for CHX mouthwash, which could be used for a while. The present study's findings indicate that CPC without alcohol is helpful in decreasing clinical periodontal markers; however, we believe that further research is required to back up this finding with strong data.

5. Conclusions
The study discovered that using a therapeutic CPC mouth rinse twice a day helps to control plaque and gingivitis or periodontitis in addition to mechanical plaque prevention, but not as much as AFCHX oral-rinse. The number of
adverse reactions (taste alterations and tooth discoloration) were associated with chlorhexidine treatment in the CHX group. Thus, similar to the findings of the current study and other studies, it can be expected that CPC oral rinses without alcohol can be another CHX mouthwash that could be used for a period of time.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors’ contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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