Clinical Effects of Chlorhexidine 0.2% and Cetylpyridinium 0.05% Combination in Comparison with Chlorhexidine, Cetylpyridinium and Persica in Reducing Oral Bacteria in Healthy Individuals

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Received: 2020-02-15, Revised: 2020-05-09, Accepted: 2020-05-09, Published: 2020-09-30

Keywords: Chlorhexidine; Cetylpyridinium; Persica; Gingivitis; Periodontitis; Oral Bacteria

Background: Preparation of a new product with the goal of reducing chlorhexidine’s side effects without decreasing (and even increasing) its effectiveness is a desirable goal for researchers in the field of oral hygiene. The aim of this study was to evaluate the efficacy of Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination in reducing oral bacteria in comparison with Chlorhexidine 0.2%, Cetylpyridinium 0.05% and Persica mouthwashes.

Methods: 100 healthy volunteers aged between 18 and 30 years were randomly assigned to 5 groups. The first group received Chlorhexidine 0.2%, the second group received Cetylpyridinium 0.05%, the third received Persica, the fourth received Chlorhexidine 0.2% plus Cetylpyridinium 0.05%, and the fifth group received Chlorhexidine 0.05% plus Cetylpyridinium 0.05%. Samples were obtained at baseline and thirty minutes after oral rinsing with the mouthwashes. The number of colony-forming units (CFU/mL) before and after mouthwash administration was compared for each sample.

Results: The preparation with the most bacterial count reduction was found to be Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination. However, the difference between efficacy of Chlorhexidine 0.2% plus Cetylpyridinium 0.05% and Chlorhexidine 0.05% plus Cetylpyridinium 0.05% was found not to be statistically significant.

Conclusion: A new mouthwash preparation including chlorhexidine 0.05% and cetylpyridinium 0.05% combination is the most desirable due to the increased efficacy and fewer side effects.

ARTICLE INFO

Article type: Original article

ABSTRACT

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Conclusion: A new mouthwash preparation including chlorhexidine 0.05% and cetylpyridinium 0.05% combination is the most desirable due to the increased efficacy and fewer side effects.

J Pharm Care 2020; 8(3): 116-122.

Please cite this paper as:
Yarahmadi N, Hashemian F, Hosseini Doust R. Clinical Effects of Chlorhexidine 0.2% and Cetylpyridinium 0.05% Combination in Comparison with Chlorhexidine, Cetylpyridinium and Persica in Reducing Oral Bacteria in Healthy Individuals. J Pharm Care 2020; 8(3): 116-122.

Introduction

Dental plaque and multispecies oral biofilms are known to play a major role in pathogenesis of periodontal diseases (1). Periodontitis is reported to be one of the most common chronic infections in adults (2). According to the systematic analysis for the global burden of disease study 2017, for all ages and both sexes, globally, in 2017, the three most common causes at Level 3 of the global burden of disease cause hierarchy in terms of all-age prevalent cases were oral disorders (3·07 billion, 2·90–3·27), and tuberculosis including latent tuberculosis infection (1·93 billion, 1·71–2·20) (3).

Global age-standardized prevalence rankings remained unchanged for the top two Level 3 causes in the global burden of disease hierarchy from 1990 to 2017, with oral disorders and headache disorders remaining the two most common causes (3). Periodontitis has been associated with increased inflammation in the body, as indicated by elevated levels of C-reactive protein (4, 5). Thus, it may relate to increased risk of myocardial infarction (6), stroke...
Primary measure for dental plaque control is mechanical cleaning by using toothbrush and dental floss (10, 11). However, mechanical methods may not be sufficient to prevent formation of dental plaque probably due to the fact that certain areas of oral cavity may not be reached by toothbrush and dental floss (12) and all individuals may not be consistent in using mechanical preventive methods (13). Thus, it is advised to use mouthwashes containing chemotherapeutic agents as an adjunct to mechanical cleaning in order to maintain an effective level of plaque control (14, 15).

Various mouthwash formulations containing antimicrobials such as Chlorhexidine, Cetylpyridinium, Triclosan (16, 17), and Persica (18) are found to be effective in reducing dental plaque and maintaining oral hygiene. Chlorhexidine with the chemical structure of two 4-chlorophenyl rings and two biguanide groups, linked by a central hexamethylene chain is known to exhibit both bacteriostatic and bactericidal properties (19). Mechanism of action of chlorhexidine is known to be increasing bacterial cell membrane’s permeability which further leads to bacterial cytoplasm precipitation and subsequent cellular death (19). Cetylpyridinium, another antiseptic agent which is widely used in mouthwash formulations is a cationic quaternary ammonium compound (20) and acts by disrupting the bacterial membrane function, causing leakage of cytoplasm and ultimate collapse of intracellular equilibrium (21, 22). A number of studies have shown efficacy of cetylpyridinium in prevention of dental plaque formation and gingivitis (23-25). Moreover, cetylpyridinium is known to be soluble in water and alcohol which makes it a desirable agent in formulations (26). Moreover, one of the most effective herbal mouthwashes, Persica (Poursina Company, Tehran, Iran), which is derived from *Salvadora Persica* has shown antimicrobial effects and efficacy against dental plaque formation in several studies (27-29).

Despite wide range of available mouthwash formulations, chlorhexidine is still widely accepted as the most effective antimicrobial agents in preventing plaque formation and gingivitis (30-32). However, chlorhexidine produces side effects such as staining of the teeth and oral mucosa and unpleasant taste (33-35) which is not acceptable for many patients. Thus, preparation of a new mouthwash combination with the goal of reducing chlorhexidine’s side effects (staining and taste disturbance) without decreasing (and even increasing) its effectiveness would be a desirable goal for researchers in the field of oral hygiene. Therefore, the aim of the present study was to evaluate the efficacy of chlorhexidine 0.2 % and cetylpyridinium 0.05 % combination in reducing oral bacteria in comparison with Chlorhexidine 0.2%, Cetylpyridinium 0.05% and Persica mouthwashes. To authors’ knowledge, to date, no one has conducted a similar comparative study. Hopefully the findings of the present study pave the way for preparation of novel antibacterial mouthwash combinations with better efficacy and lower side effects.

**Methods**

The present study was conducted at Microbiology Lab of Tehran Medical Sciences, Islamic Azad University in 2015. Hundred healthy volunteers who were students of Tehran Medical Sciences, Islamic Azad University and aged between 18 and 30 years were enrolled in the present study. Inclusion criteria comprised healthy volunteers of both genders who had no active oral infections, no history of known hypersensitivity to any of the ingredients of the mouthwashes, were not treated in the last three months with antibiotics for dental pathology and had not undergone orthodontic procedures in the past. Exclusion criteria comprised pregnancy, lactation, smoking, any chronic diseases, history of alcohol or drug abuse or participation in other clinical studies in the last 4 weeks. The study protocol was approved by the Ethics Committee of Tehran Medical Sciences, Islamic Azad University (Ref No.: 1156) and performed in accordance with the Helsinki Declaration of 1975, revised in 2000. All participants were informed of the study procedure and signed written consent forms prior to the study. Sample size selection was done according to previous clinical studies of Chlorhexidine and Cetylpyridinium mouthwashes (36, 37).

Randomization was done using a computer generated random allocation table assigning the participants into 5 groups of 20 individuals. Moreover, computer generated random sequence was used to assign each group to one of the mouthwash preparations. Thus, the first group received Chlorhexidine 0.2% without alcohol, the second group received Cetylpyridinium 0.05%, the third group received Persica (Poursina Company, Tehran, Iran), the fourth group received Chlorhexidine 0.2% plus Cetylpyridinium 0.05%, and the fifth group received Chlorhexidine 0.2% plus Cetylpyridinium 0.05%. Each test solutions were provided in a container labeled with a code and could not be identified by the investigator or the participant. The participants did not eat anything two hours prior to sampling. Samples were obtained from mesial, distal, vestibular, and lingual sides of all teeth at baseline and thirty minutes after the administration of the mouthwashes. The participants were asked to rinse their oral cavity with the provided mouthwash for 30 seconds and not to eat or rinse their mouth for thirty minutes after administration of the mouthwash.

Primary outcome measure was the mean change in oral bacterial counts across different treatment groups. The secondary outcome was detecting possible side effects. The collected samples were transferred to microtubes containing 500 mL of Normal Saline. Samples obtained were diluted in normal saline and subsequently, 1/1, ¼ dilutions were made. 50 microliters from each dilution were diluted in normal saline and subsequently, 1/1, ½, ¼ dilutions were made. 50 microliters from each dilution were cultured on Brucella agar media. After 24 hours of incubation at 37°C, colony counts were determined, and the number of colony-forming units (CFU/mL) before and after administration of mouthwash was compared for each
sample using a colony counter.

The obtained data was analyzed by SPSS 18.0 software. Analysis of variance (ANOVA) was used to compare the baseline microbiological results between the treatment groups. Moreover, paired T-tests were used to make intergroup comparisons between baseline and each post-treatment.

**Results**

T100 healthy volunteers (with age range of 18-30 years) who met the inclusion criteria were included in the study. Demographics of the patients are shown in Table 1. No statistically significant difference was found between the groups regarding age, gender and smoking status prior to the study (p = 0.903).

**Table 1.** Demographics of the participants.

| Variables           | No. (%) of Participants |
|---------------------|-------------------------|
| Gender              |                         |
| Female              | 59 (59%)                |
| Male                | 41 (41%)                |
| Age                 |                         |
| 18-21               | 42 (42%)                |
| 21-24               | 29 (29%)                |
| 24-27               | 17 (17%)                |
| 27-30               | 12 (12%)                |
| Marital Status      |                         |
| Single              | 72 (72%)                |
| Married             | 28 (28%)                |
| Smoking             |                         |
| Smoker              | 0                       |
| Non-Smoker          | 98 (98%)                |
| Former Smoker       | 2 (2%)                  |
| Education           |                         |
| Diploma             | 16 (16%)                |
| Master of Sciences/ Master of Arts | 6 (6%)          |
| Doctorate           | 3 (3%)                  |
| Doctorate Student   | 75 (75%)                |

\(n=100\)

**Table 2.** Comparison of the bacterial count before and after use of mouthwashes.

|          | Sum of Squares | df | Mean Square | F    | Sig. |
|----------|----------------|----|-------------|------|------|
| Before   | Between Groups | 27627.140 | 4  | 6906.785 | .260 | .903 |
|          | Within Groups  | 2524267.500 | 95 | 26571.237|      |      |
|          | Total          | 2551894.640 | 99 |        |      |      |
| After    | Between Groups | 1924.110  | 4  | 481.028 | 41.229| .000 |
|          | Within Groups  | 1108.385  | 95 | 11.667  |      |      |
|          | Total          | 3032.495  | 99 |        |      |      |
Results of ANOVA indicated a significant decrease in colony count of all 5 groups from baseline (P< 0.05) (Table 2). The group with the most reduction in oral bacteria count was found to be Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination. However, results of T-tests showed no statistically significant difference between combination of Chlorhexidine 0.2% and Cetylpyridinium 0.05% and combination of Chlorhexidine 0.05% and Cetylpyridinium 0.05% regarding efficacy. Additionally, no statistically significant difference was found between efficacy of Chlorhexidine 0.2% and Cetylpyridinium 0.05%. Descriptive results showing oral bacteria count before and after use of mouthwashes are shown in Table 3. Comparisons of mean oral cavity bacteria counts before and after use of the mouthwashes are shown in Figure 1. No significant side effects were observed.

Table 3. Descriptive results of oral bacteria count before and after use of mouthwashes.

|       | N  | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | Min  | Max  |
|-------|----|------|----------------|------------|---------------------------------|------|------|
|       |    |      |                |            | Lower Bound                      |      |      |
|       |    |      |                |            | Upper Bound                      |      |      |
| Before| A  | 20   | 308.50         | 163.31291  | 36.51788                        | 232.0672 | 384.9328 |
|       |    |      |                |            |                                 | 69.00 | 593.00|
|       | B  | 20   | 311.40         | 166.05592  | 37.13123                        | 233.6834 | 389.1166 |
|       |    |      |                |            |                                 | 59.00 | 685.00|
|       | C  | 20   | 350.70         | 182.90495  | 40.89879                        | 265.0978 | 436.3022 |
|       |    |      |                |            |                                 | 111.00 | 814.00|
|       | D  | 20   | 314.65         | 137.12778  | 30.66270                        | 250.4722 | 378.8278 |
|       |    |      |                |            |                                 | 121.00 | 563.00|
|       | E  | 20   | 337.55         | 162.33379  | 36.29894                        | 261.5754 | 413.5246 |
|       |    |      |                |            |                                 | 97.00  | 582.00|
|       | Total| 100 | 324.56         | 160.55128  | 16.05513                        | 292.7031 | 356.4169 |
| After | A  | 20   | 173.15         | 112.22078  | 25.09333                        | 120.6291 | 225.6709 |
|       |    |      |                |            |                                 | 21.00  | 436.00|
|       | B  | 20   | 32.75          | 15.47791   | 3.46997                         | 25.5061  | 39.9939 |
|       |    |      |                |            |                                 | 10.00  | 72.00 |
|       | C  | 20   | 35.75          | 20.08895   | 4.49203                         | 26.3481  | 45.1519 |
|       |    |      |                |            |                                 | 11.00  | 77.00 |
|       | D  | 20   | 212.65         | 105.61363  | 23.61593                        | 163.2213 | 262.0787 |
|       |    |      |                |            |                                 | 55.00  | 408.00|
|       | E  | 20   | 284.04         | 145.64249  | 32.56665                        | 215.8872 | 352.2128 |
|       |    |      |                |            |                                 | 66.00  | 510.00|
|       | Total| 100 | 147.67         | 136.71936  | 13.67194                        | 120.5419 | 174.7981 |

A: Chlorhexidine 0.2%, B: Chlorhexidine 0.2% and Cetylpyridinium 0.05%, C: Chlorhexidine 0.05% and Cetylpyridinium 0.05% D: Cetylpyridinium 0.05%, E: Persica.

Figure 1. Mean oral cavity bacterial counts before and after use of the mouthwashes.
Discussion

Several studies have investigated the efficacy of different mouthwash formulations on oral cavity bacterial and plaque formation reduction (2, 38-41). Yet, Chlorhexidine is still widely accepted as the most effective antimicrobial agents in preventing plaque formation (30-32). However, chlorhexidine’s side effects including staining of the teeth and oral mucosa and unpleasant taste sometimes limits its application (33-35). Thus, the aim of the present study was to evaluate the efficacy of Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination in reducing oral bacteria in comparison with Chlorhexidine 0.2%, Cetylpyridinium 0.05% and Persica mouthwashes. According to our results, Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination showed the most reduction in oral bacterial counts compared to the other treatment groups. In the second rank, Chlorhexidine 0.05% and Cetylpyridinium 0.05% combination was reported to be more effective in bacterial reduction compared with chlorhexidine 0.2%, Cetylpyridinium 0.05% and Persica alone. To authors’ knowledge, to date, no one has conducted such a comparative study and authors believe that the results of the present study give insight and hopefully pave the way for preparation of new mouthwash combinations with the goal of reducing chlorhexidine’s side effects (staining and taste disturbance) without decreasing (and even increasing) its effectiveness.

In a randomized, double-blind, cross over study, Bascones et al., evaluated the effects of adding either sodium fluoride 0.05% or cetylpyridinium 0.05% to chlorhexidine 0.12% on levels of gingivitis, dental plaque, supragingival calculus, and dental staining in a 21 treatment period. Their results showed a significant increase in plaque index in the group receiving chlorhexidine 0.12% plus sodium fluoride 0.05% treatment in comparison with groups receiving chlorhexidine 0.12% alone or combination of chlorhexidine 0.12% and cetylpyridinium 0.05%. Moreover, a significant increase in supragingival calculus was observed in the chlorhexidine 0.12% + sodium fluoride 0.05% treatment group in comparison with the other groups. Thus, it seems that adding sodium fluoride 0.05% to chlorhexidine 0.12% may not increase efficacy in terms of reducing plaque index. The mentioned combination most probably doesn’t reduce side effects as well. Additionally, tongue staining was more frequently observed in the group receiving chlorhexidine 0.12% and cetylpyridinium 0.05% combination mouthwash. However, not in line with the results of the present study, they concluded that adding either sodium fluoride 0.05% or cetylpyridinium 0.05% to chlorhexidine 0.12% probably does not make significant differences in the efficacy of the mouthwash and may even reduce its effectiveness and even increase incidences of tongue staining (19).

In a similar study, Quirynen et al., assessed the efficacy of chlorhexidine 0.2%, chlorhexidine 0.12%, chlorhexidine 0.12% + sodium fluoride 0.05%, and chlorhexidine 0.12% + cetylpyridinium 0.05% in terms of plaque reduction and side effects. Their findings were almost consistent with results obtained from Bascones et al.’s study. Mouthwash formulations containing chlorhexidine 0.12% and chlorhexidine 0.12% + cetylpyridinium 0.05% were found to demonstrate similar efficacy as chlorhexidine 0.2% formulations in dental plaque reduction. Moreover, chlorhexidine 0.12% + sodium fluoride 0.05% showed smaller clinical efficacy in comparison with other treatment groups. Additionally, subjective ratings for chlorhexidine combination formulations were found to be better especially in terms of taste (43). Therefore, one may argue that preparation of lower concentrations of chlorhexidine combinations most probably exhibit more efficacy and better patient compliance together with less adverse effects.

Gründemann et al., conducted a study in order to compare the efficacy of chlorhexidine and combination of chlorhexidine and an oxidizing mouth rinse like peroxyborate in reducing plaque formation, gingivitis and staining. They found that combination of peroxyborate and chlorhexidine was significantly more effective in reducing plaque formation, gingivitis, and staining (44). In a double-blind cross over study, Franco Neto et al., compared the effects of chlorhexidine 0.12% and chlorhexidine 0.2% on plaque formation and gingival bleeding. Their results showed no significant differences between the two chlorhexidine concentrations in reducing plaque formation and gingival bleeding (45). Similarly, in the present study, no significant difference was found between chlorhexidine 0.2% + cetylpyridinium 0.05% combination and chlorhexidine 0.05% + cetylpyridinium 0.05% combination regarding efficacy.

In a similar placebo-controlled study, Najafi et al., found no significant differences in plaque index and gingival index reduction between chlorhexidine 0.12% and chlorhexidine 0.2% groups. Moreover, the results showed much more dental staining with chlorhexidine 0.2% in comparison with chlorhexidine 0.12% (46). Thus, the results of their study were in favor of preparation of lower concentrations of chlorhexidine combinations due to similar efficacy and less adverse effects. Mozafari et al., compared antibacterial and cytotoxic effects of chlorhexidine and Persica. Persica was found to be inferior to chlorhexidine in terms of antibacterial activity. Moreover, Persica was found to be less toxic than chlorhexidine (47). In a recent pilot study, effects of two newly-formulated chlorhexidine and Cetylpyridinium mouthwashes (0.12% chlorhexidine and 0.05% Cetylpyridinium versus 0.03% chlorhexidine and 0.05% Cetylpyridinium) following scaling and root planning was evaluated. Consistent with the results of the present study, the newly formulated 0.12% chlorhexidine and 0.05% CPC mouthrinse showed larger plaque level reductions, without showing more adverse effects (48).

According to the present study, chlorhexidine 0.2 % and cetylpyridinium 0.05 % combination showed the most reduction in oral bacterial counts in comparison with the other treatment groups. In the second rank, chlorhexidine 0.05 % and cetylpyridinium 0.05 % combination was found to be more effective in bacterial reduction in comparison
with chlorhexidine 0.2%, Cetylpyridinium 0.05% and Persica alone. However, no statistically significant difference was found between chlorhexidine 0.2% + cetylpyridinium 0.05% and chlorhexidine 0.05% + cetylpyridinium 0.05% regarding efficacy. Thus, preparation of chlorhexidine 0.05% and cetylpyridinium 0.05% combination which exhibited more efficacy and probably demonstrates less adverse effects (staining and unpleasant taste) due to lower concentrations is recommended.

Acknowledgment

Academic research fund was provided by Tehran Medical Sciences, Islamic Azad University. It is important to note that Tehran Medical Sciences, Islamic Azad University is a non-governmental non-profit organization. The authors wish to thank Dr Elnaz Roohi for her insightful comments in reviewing and editing of the manuscript.

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