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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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ABSTRACT

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.

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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 (3·47 billion, 95% UI 3·27–3·68), headache 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

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(7), and atherosclerosis (8, 9).

Primary measure for dental plaque control is mechanical cleaning by using tooth brush 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 0.05% 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, 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[a]
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%)

a 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			

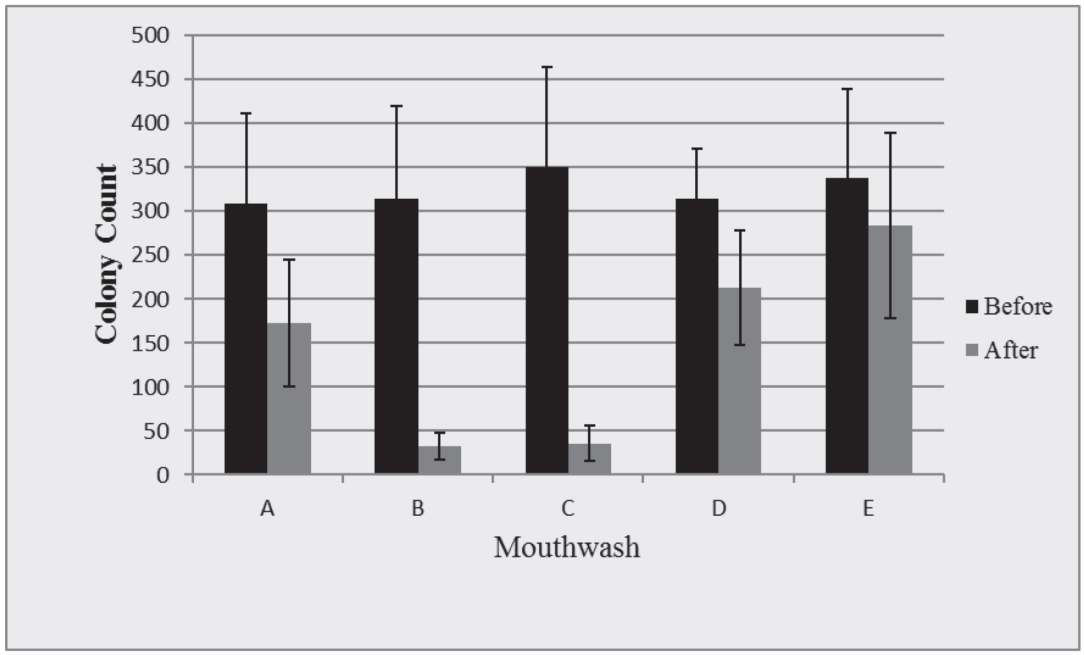
Results of ANOVA indicated a significant decrease in colony count of all 5 groups from baseline (P< 0.05) (Table 2).
The group with the most reduction in oral bacteria count was found to be Chlorhexidine 0.2% and Cetylpyridinium 0.05% combination. However, results of T-tests showed no statistically significant 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	59.00	814.00
After	A	20	173.15	112.22078	25.09333	120.6291	225.6709	21.00	436.00
	B	20	32.75	15.47791	3.46097	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
	5	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	10.00	510.00

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.



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.

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, supragingivial 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 supragingivial calculus was observed in the chlorhexidine 0.12% % plus 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% chlorhexidinea 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.

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References

1. Page RC, Schröder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34(3):235–49.

2. Lösche WJ, Grossman NS. Periodontal disease as a specific, albeit chronic infection diagnosis and treatment. Clin Microbiol Rev 2001;14:727-52.

3. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392(10159):1789-1858

4. D'Aiuto F, Parkar M, Andreou G, et al. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. J Dent Res 2004;83(2):156–60.

5. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on C- reactive protein in relation to periodontitis. J Clin Periodontol 2008;35(4):277-90.

6. Pussinen PJ, Alfthan G, Tuomilehto J, Asikainen S, Jousilahti P. High serum antibody levels to Porphyromonas gingivalis predict myocardial infarction. European Journal of Cardiovascular Prevention & Rehabilitation. 2004;11:408–11.

7. Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J. Systemic exposure to Porphyromonas gingivalis predicts incident stroke. Atherosclerosis 2007;193:222–28.

8. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A Systematic Review. Annals of Periodontology 2003;8:38–53.

9. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal Disease and Coronary Heart Disease Incidence: A Systematic Review and Meta-analysis. Journal of General Internal Medicine 2008;23:2079–86.

10. Rosema NA, Timmerman MF, Versteeg PA, et al. Comparison of the use of different modes of mechanical oral hygiene in prevention of plaque and gingivitis. J Periodontal 2008;79(8):1386-94.

11. Van der Weijden GA, Hioe KP. A systemic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. J Clin Periodontol 2005;32(Suppl 6):214-28.

12. Sturzenburger OP, Leonard GJ. The effect of a mouthwash as adjunct in tooth cleaning. J Periodontol 1969;40(5):299-301.

13. Marsh PD. Microbiological aspects of the chemical control of plaque and gingivitis. J Dent Res 2002;71(7):1431-8.

14. Barnett ML. The role of therapeutic antimicrobial mouthrinses in clinical practice. J Am Dent Assoc 2003;134(6):699-704.

15. Barnett ML. The rationale for the daily use of an antimicrobial mouthrinse. J Am Dent Assoc 2006;137 Suppl:16S-21S.

16. Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis 2003:9(Suppl 1):23-9.

17. Williams MI. The antimicrobial and antiplaque effectiveness of mouthwashes containing cetylpyridinium chloride with and without alcohol in improving gingival health. J Clin Dent 2011;22(6):179-82.

18. Khalessi AM, Pack AR, Thomson WM, Tompkins GR. An in vivo study of the plaque control efficacy of Persica: a commercially available herbal mouthwash containing extracts of Salvadora persica. Int Dent J 2004;54(5):279-83.

19. Bascones A, Morante S, Mateos L, Mata M, Poblet J. Influence of additional active ingredients on the effectiveness of non-alcoholic chlorhexidine mouthwashes: a randomized controlled trial. J Periodontol 2005;76(9):1469-75.

20. Schäffer LM, Szewczyk G, Nesta J, et al. In vitro antibacterial efficacy of cetylpyridinium chloride-containing mouthwashes. J Clin Dent 2011;22(6):183-6.

21. Sheie A. Modes of action of currently known chemical anti-plaque agents other than chlorhexidine. J Dent Res 1989;68:1609-16.

22. Haps S, Slot DE, Berchier CE, Van der Weijden GA. The effects of cetylpyridinium chloride-containing mouth rinses as adjuncts to toothbrushing on plaque and parameters of gingivial inflammation: a systematic review. Int J Dent Hyg 2008;6(4):290-303.

23. Allen DR, Davies R, Bradshaw B, et al. Efficacy of a mouthrinse containing 0.05% cetylpyridinium chloride for the control of plaque and gingivitis: a 6-month clinical study in adults. Compend Contin Educ Dent 1998;19(2 Suppl):20-6.

24. Rawlinson A, Pollington S, Walsh TF, et al. Efficacy of two alcohol-free cetylpyridinium chloride mouthwashes- a randomized double-blind cross over study. J Clin Dent 2011;22(6):200-3.

25. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and

antigingivitis agents. J Am Dent Assoc 2006;137(12):1649-57.

26. He S, Wei Y, Fan X, Hu D, Screenivasan PK. A clinical study to assess the 12-hour antimicrobial effects of cetylpyridinium chloride mouthwashes on supragingivial plaque bacteria. J Clin Dent 2011;22(6):195-9.

27. Moeintaghavi A, Arab H, Khajekaramodini M, Hosseini R, Danesteh H, Niknami H. In vitro antimicrobial comparison of chlorhexidine, persica mouthwash and miswak extract. J Contemp Dent Pract 2012;13(2):147-52.

28. Halawany HS. A review on miswak (Salvadora persica) and its effect on various aspects of oral health. Saudi Dent J 2012;24(2):63-9.

29. Al-Lafi T, Abadneh H. The effect of the extract of the Miswak (chewing sticks) used Jordan and the Middle East on oral bacteria. Int Dent J 1995;45(3):218-22.

30. Renton-Harper P, Addy M, Moran J, Doherty FM, Newcombe RG. A comparison of chlorhexidine, cetylpyridinium, triclosan, and C31G mouthrinse products for plaque inhibition. J Periodontol 1996;67(5):486-9.

31. Elworthy A, Greenman J, Doherty FM, Newcobe RG, Addy M. The substantivity of a number of oral hygiene products determined by the duration of effects on salivary bacteria. J Periodontol 1996;67(6):572-6.

32. Leyes Borajo JL, Garcia VL, Lopez CG, Rodriquez-Nunez I, Garcia FM, Gallas TM. Efficacy of chlorhexidine mouthrinses with and without alcohol: a clinical study. J Periodontol 2002;73(3):317-21.

33. Ernst CP, Prockl K, Willershausen B. The effectiveness and side effects of 0.1% and 0.2% chlorhexidine mouthrinses: A clinical study. Quintessence Int 1998;29(7):443-8.

34. Berchier CE, Slot DE, Van der Weijden GA. The efficacy of 0.12% chlorhexidine mouthrinse compared with 0.2% on plaque accumulation and periodontal parameters: a systemic review. J Clin Periodontol 2010;37(9):829-39.

35. Addy M, Mahdavi SA, Lyon T. Dietary staining in vitro by mouthrinses as a comparative measure of antiseptic activity and predictor of sating in vivo. J Dent 1995;23(2):95-9.

36. Haydari M, Bardakci AG, Koldsland OC, Aass AM, Sandvik L, Preus HR. Comparing the effect of 0.06% -, 0.12% and 0.2% Chlorhexidine on plaque, bleeding and side effects in an experimental gingivitis model: a parallel group, double masked randomized clinical trial. BMC Oral Health 2017; 17(1):118.

37. Pulcini A, Bollaín J, Sanz-Sánchez I, et al. Clinical effects of the adjunctive use of a 0.03% chlorhexidine and 0.05% cetylpyridinium chloride mouth rinse in the management of peri-implant diseases: A randomized clinical trial. J Clin Periodontol 2019;46 (3):342-53.

38. Lobene RR. Clinical studies of plaque control agents: an overview. J Dent Res 1979;58(12):2381-8.

39. Finne DH, Furgang D, Barnett ML. Comparative antimicrobial activities of antiseptic mouthrinses against isogenic planktonic and biofilm forms of Actinobacillus actinomycetmcomitans. J Clin Periodontol 2001;28(7):697-700.

40. Pan PC, Harper S, Ricci-Nittel D, Lux R, Shi W. In vitro evidence for the efficacy of antimicrobial mouthrinses. J Dent 2010;38(Suppl 1):S16-20.

41. DePaola LG, Spolarich AE. Safety and efficacy of antimicrobial mouthrinses in clinical practice. J Dent Hyg 2007;81(5):1-16.

42. Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol 2005;99(4):703-15.

43. Quirynen M, Avontroodt P, Peeters W, Pauwels M, Coucke W, Van Steenberghe D. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. J Cli Periodontol 2001;28(12):1127-36.

44. Gründemann LJ, Timmerman MF, Ijzerman Y,et al. Stain, plaque and gingivitis reduction by combining chlorhexidine and peroxyborate. J Clin Periodontol 2000;27(1):9–15.

45. Franco Neto CA, Fatturi Parolo CC, Rösing CK, Maltz M. Comparative analysis of the effect of two chlorhexidine mouthrinses on plaque accumulation and gingival bleeding. Braz Oral Res 2008;22(2):139-44.

46. Najafi MH, Taheri M, Mokhtari MR, et al. Comparative study of 0.2% and 0.12% digluconate chlorhexidine mouth rinses on the level of dental staining and gingival indices. Dent Res J (Isfahan) 2012;9(3):305-8.

47. Mozaffari B, Mansori SH, Rajabalian S, Alimardani A, Mohamadi M. In vitro study between antibacterial and cytotoxic effects of chlorhexidine and persica mouthrinses. Journal of Dental School 2005;23(3):494-509.

48. García-Gargallo M, Zurlohe M, Montero E, et al. Evaluation of new chlorhexidine- and cetylpyridinium chloride-based mouthrinse formulations adjunctive to scaling and root planing: pilot study. Int J Dent Hyg 2017;15(4):269-79.