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W Papaioannou
S Vassilopoulos
I Vrotsos
V Margaritis
V Panis

Authors' affiliations:
W Papaioannou, Department of Preventive and Community Dentistry, School of Dentistry, National and Kapodistrian University of Athens, Athens, Greece
S Vassilopoulos, *I Vrotsos*, *V Panis*, Department of Periodontology, School of Dentistry, National and Kapodistrian University of Athens, Athens, Greece
V Margaritis, College of Health Sciences, Walden University, Minneapolis, MN, USA

Correspondence to:
W. Papaioannou
Department of Preventive and Community Dentistry
School of Dentistry
National and Kapodistrian University of Athens
2 Thivon Str.
115 27 Athens
Greece
Tel.: +30 210 7461210
Fax: +30 210 7461132
E-mail: vpapaio@dent.uoa.gr

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A comparison of a new alcohol-free 0.2% chlorhexidine oral rinse to an established 0.2% chlorhexidine rinse with alcohol for the control of dental plaque accumulation

Abstract: *Objectives:* To compare the clinical efficacy of two formulations (alcohol and alcohol free) of 0.2% chlorhexidine (CHX) rinses on plaque, gingivitis and discoloration of teeth. *Methods:* This double-blind crossover study consisted of one group of 10 volunteer dental students that followed two 21-day experimental gingivitis test periods. During these periods, the subjects abstained from oral hygiene except for the oral rinse provided. The study started after an initial two-week preparation programme that included a professional prophylaxis and repeated oral hygiene instructions. This was repeated for the 14-day washout period between the two rinses, including prophylaxis as per the first stage of the study. A calibrated examiner performed the clinical measurements at the beginning (baseline) and end of each study stage. The presence and amount of plaque were recorded using the Silness and Löe plaque index (PI) and gingival inflammation by the gingival index (GI) while the discoloration index (DI) was recorded on the buccal and lingual surfaces of the six anterior teeth of both the mandible and maxilla. *Results:* Mean PI increased similarly for both solutions; however, the differences between initial and final values were statistically significant only for CHLOREL®. Similarly, the mean values for the GI showed small increases over the course of the study periods, but not statistically significant for either solution. The mean DI increased significantly for both solutions. Regarding the comparison of the initial and final values between the solutions, *per* index, no statistically significant differences were observed. *Conclusion:* The non-alcoholic chlorhexidine rinse had comparable levels of action as the generally recognized gold standard alcoholic rinse.

Key words: chlorhexidine mouthrinse; dental biofilm; gingivitis; staining

Introduction

The determining role of plaque in the aetiopathogenesis of periodontal diseases has been adequately shown in the literature (1). Plaque build-up is highly related to the presence of gingivitis which, if remains untreated, may lead to non-reversible periodontal destruction in susceptible patients (2). On the other hand, plaque control using mechanical means (toothbrush and interdental cleaning aids), when practiced successfully and on a daily basis, is usually sufficient for the preservation of healthy dental

and periodontal tissues (3). The majority of patients, however, do not succeed in effectively removing plaque, especially in the interdental areas and other hard-to-reach surfaces. This is because they do not spend enough time for their oral hygiene (usually less than the widely proposed 2 min) and also due to the lack of skill that may be a result of indifference or lack of essential knowledge on how to properly use mechanical hygiene means (4). Therefore, the complementary use of antiseptics in the form of mouthwashes has been examined for use on a daily basis and has been proven to be helpful in successfully controlling plaque and gingival inflammation (5).

Chlorhexidine digluconate (CHX) is a powerful antimicrobial substance that chemically belongs to the bisguanides family (ATC Code: A01AB03). Mouthwashes that contain CHX in different concentrations (0.1-0.2%) are considered to be the most effective in plaque and gingival inflammation reduction (6, 7). This is due to the powerful antimicrobial action of CHX, which primarily strikes the bacterial cell membrane causing leakage of cell components of Gram-positive bacteria, Gram-negative bacteria, fungi and viruses (HSV1, HSV2, Influenza A) (8). CHX can adhere to the bacteria, preventing their adhesion to the dental surfaces. By its adhesion to salivary proteins, it inhibits the formation of the biofilm. Moreover, it penetrates into the plaque biofilm and acts against the already incorporated bacteria (1). CHX preserves its antimicrobial action for more than 12 h due to its supragingival substantivity. It has both a bactericidal and bacteriostatic effect dependent on the available concentration. It does not cause the creation of resistant microbial strains, even after prolonged use (9). In clinical studies lasting less than 6 months, CHX succeeded in reducing plaque concentration by 48–61% and inflammation by 27–67% (10).

The most frequent side effect after prolonged CHX use is the occurrence of discoloration of the dental surfaces and the tongue. Moreover, there is frequent reference to the increase in rate of calculus formation. In cases of hypersensitivity or improper use of CHX (increased amount and/or frequency of washes, prolonged holding of the solution in the mouth), damage to the gingiva and mucosa may be observed, which subsides after termination of use. More uncommon effects are an allergic reaction or the swelling of the parotid gland, as well as taste alterations usually encountered with salty foods (11).

Aim

The aim of this study was to compare the clinical efficacy of two formulations, both of which contain the same concentration of active ingredient in the solution (CHX 0.2% w/v) but have different formulation excipients, on a) the formation of plaque, b) gingival inflammation and c) the discoloration of the dental tissues.

Materials and methods

A double-blind crossover study based on the 21-day experimental gingivitis model (12) was designed to examine the effect of the two rinses.

Study population

The subjects taking part in the study were 10 volunteers; undergraduate students of the National and Kapodistrian University of Athens School of Dentistry. These subjects were healthy, non-smokers, with high level of oral health (Community Periodontal Index <2) (13), with no active dental caries and with no removable dental prostheses or fixed or removable orthodontic appliances. Subjects taking medication were not included as well as those with history of allergy to any of the ingredients of the investigated mouthwash solutions. There was no exclusion from the study on the basis of gender or nationality. Ethical approval was obtained from the *Research and Ethics Committee* of the School of Dentistry, National and Kapodistrian University of Athens. The subjects who satisfied the study criteria were asked to sign an informed consent form.

Clinical examination

The clinical measurements were performed by a calibrated examiner at the beginning (baseline) and at the end of each study stage. The examiner was blinded to the solution used as well as to the previous measurements. An assistant recorded the findings. The presence and the amount of plaque were recorded using the Silness and Löe plaque index (PI) (14). More specifically, this index was measured on the mesial, middle and distal of both the buccal and lingual surface of all teeth except for the third molar and with a 0–3 gradation (0 = absence of plaque, 1 = no visible plaque detected by periodontal probe, 2 = moderate accumulation along the gingival margin of the tooth, 3 = abundant accumulation on the gums and on the dental surface).

On the same surfaces and with the same 0–3 grading, gum inflammation was also assessed with the help of the gingival index (GI) by Löe and Silness (15) (0 = lack of inflammation, 1 = light discoloration and light swelling but lack of bleeding during probing, 2 = redness, swelling and bleeding during probing, 3 = intense redness, swelling and tendency to bleed automatically).

Finally, the discoloration index (DI) was recorded on the buccal and lingual surfaces directly without the use of photographs, for the six anterior teeth of both the mandible and maxilla. This index records the discoloration both qualitatively (colour intensity) and quantitatively (amount) (16). More specifically:

Intensity grading

0 = lack of stain, 1 = light stain – yellow to brown, slightly visible, 2 = medium stain – medium brown colour, 3 = dark stain – dark brown to black colour.

Amount grading

0 = lack of stain, 1 = thin stain line (<1 mm width), 2 = moderate band of stain (1–2 mm), 3 = wide band of stain (>2 mm).

Both of these scores are combined into a single overall score according to the formula:

1.5 × stain intensity + 1 × stain amount, resulting in a final DI rate for the mouth, which was a mean of all examined surfaces.

The formula was developed taking into consideration that even a small amount of black stain can be more aesthetically annoying for the patient rather than a wider amount of light discoloration.

Experimental protocol

The 10 volunteers followed a two-week preparation programme that included plaque removal through a professional prophylaxis – as thoroughly as possible – and repeated instructions on oral hygiene. The objective was that the subjects taking part in the study were free of microbial plaque and gingivitis at the end of this time period. This study consisted of only one group that followed two 21-day experimental gingivitis test periods. During these time periods, the study subjects abstained from every kind of oral hygiene with mechanical or other means except by the oral rinse provided. The products under investigation, CHLOREL® 0.2% w/v (Intermed S.A., Kifissia, Greece) and CORSODYL® 0.2% w/v Mint Mouthwash (GlaxoSmithKline Consumer Healthcare, Brentford, UK), were given to the researchers by Intermed S.A., and subsequently to the study subjects at the respective time period, in identical packaging with only the following indications: Bottle A, Bottle B. The 10 volunteers rinsed every morning and evening and for a duration of 1 min with a) 10 ml solution from Bottle A for period 1 and b) 10 ml solution from Bottle B for period 2. This was a double-blind study. The contents of the bottles were revealed to the investigators after completion of the study.

After the end of the first test period, a 14-day washout period followed, during which the study subjects resumed oral hygiene with mechanical means at home, while plaque removal, tooth scaling and polishing were repeated at the clinic. Both at the beginning and at the end of each test period, the same examiner obtained and analysed the clinical measurements.

Briefly, the stages were the following:

1 Initial clinical measures (Day 0 – Baseline: PI, GI, DI, CPI)
2 Two-week preparation programme:

Repeated instructions on oral hygiene, plaque removal, tooth scaling and polishing at the clinic.

3 1st test period lasting 3 weeks: clinical examinations at the start (PI, GI, DI)

The subject abstains from all means of oral hygiene; rinses every morning and evening with 10 ml of solution A for 1 min.

Clinical examinations repeated (PI, GI, DI) at the end of the period.

4 Washout period and 14-day preparation:

The use of mouthwash is ended and daily oral hygiene using mechanical means is started.

Repeated instructions on oral hygiene, plaque removal, tooth scaling and polishing.

5 2nd test period lasting 3 weeks: clinical examinations at the start (PI, GI, DI)

The subject abstains again from all means of oral hygiene; rinses every morning and evening with 10 ml of solution B for 1 min.

Clinical examinations repeated (PI, GI, DI) at the end of the test period.

6 Completion of study: subjects have plaque removed by scaling and polishing at the clinic.

Statistical methodology

Sample size estimation was performed by calculating the effect size (Cohen's d) based on a similar study by Solis and coworkers (17): according to the data of this study, the effect size was d = 1.15; thus, for a = 0.05 and adequate statistical power (power >0.80), 10 subjects are required *per* intervention group. Due to the non-normal distribution of the frequency of the indices, the potential differences between the means of the indices initially and after completion of the study *per* solution were investigated by Wilcoxon signed–rank test, with a significance level of a = 0.05. In addition, a comparison of the initial and final values of the solutions *per* index was performed using Mann–Whitney *U*-test. The analyses were performed using the IBM software package SPSS, v.21: IBM Corp., Armonk, NY, USA.

Results

The group of volunteers comprised six females and four male students with a mean age of 23.4 years (SD 3.9). All had very good/excellent oral health. The mean values and standard deviations of all indices/clinical parameters for both solutions, initially (the start of the test period) and finally (the end of the test period), are presented in Table 1. Mean values (standard deviations) of PI increased similarly for both solutions; however, these differences between initial and final values were statistically significant only for CHLOREL® (0.52 (0.15) to 0.75 (0.19), respectively). Similarly, the mean values for the GI showed small increases over the course of the study periods, but these differences were not found to be statistically significant for either solution. The mean values of DI for CORSODYL® and CHLOREL®, which were at 0 at the beginning of each study period increased significantly for both solutions, with the former showing the highest mean final score, that is 0.20 (0.30). These differences were statistically significant for both solutions.

Mean values (standard deviations) of the percentage of surfaces free of plaque for the solution CORSODYL® initially and finally were 52.55 (19.50) and 36.95 (18.17), respectively,

while for the solution CHLOREL® were 51.28 (11.82) and 32.62 (16.80), respectively. However, these differences were statistically significant only for CHLOREL®.

Regarding the comparison of the initial and final values between the solutions, *per* index, it can be noted that no statistically significant differences was observed. However, an indicative statistical difference ($P < 0.16$) was observed between the final mean values of the DI index for the solutions (0.20 and 0.06, for CORSODYL® and CHLOREL® respectively). Taking the relatively small sample size into consideration, this result suggests that there is a trend that has great chances to be confirmed in larger test groups.

No adverse events occurred in any of the participants during the study.

Discussion

Chlorhexidine (CHX) solutions have been shown both experimentally and clinically to provide important antibacterial effect, such as to be considered the gold standard (18, 19). This results in a significant control in plaque growth, especially in cases where there are lower levels of plaque accumulations or after plaque has been removed. The end result is the control and minimization of gingival inflammation. The first step in combating, or even more importantly preventing, periodontitis is control of this inflammation. Traditionally, the solutions used were of an alcoholic nature; specifically, ethanol was used as a solvent and stabilizer for the active and flavour ingredients. However, more recently there has been an increasing interest in alcohol-free solutions due to fears of a possible link of alcohol to oral carcinogenesis, although this link is tenuous at best for oral rinses (20, 21). An additional benefit in the use of alcohol-free solutions is the reduced risk of use in cases where alcohol ingestion is to be avoided (*i.e.* children and alcoholics) or in settings such as prisons with restricted alcohol availability (22). Nevertheless, the question concerning the clinical efficacy of the newer alcohol-free formulations remains. Moreover, by increasing the available information, clinicians and patients may choose alcohol-free solutions with greater confidence that they are not sacrificing efficacy over peace of mind for any possible unwanted effects.

In this current study, we examined the efficacy of a new alcohol-free CHX oral rinse, CHLOREL®, to an established alcohol containing CHX solution considered the gold standard in antibacterial oral rinses, CORSODYL®. Moreover, the classic experimental gingivitis model developed by Löe and co-workers (12) was chosen to have a better insight in the effect on the *de novo* plaque accumulation. Most of the more recent studies on oral rinse effectiveness opt for the 3–4 day (23, 24) or 14-day (17, 25) plaque accumulation set-ups. However, the 21-day gingivitis model is still considered as the best design to examine the effect on both plaque growth and the concomitant development of inflammation (gingivitis). Indeed, the important impact of both rinses on plaque accumulation and gingivitis was shown.

Using the same individuals in a crossover study set-up to compare the two solutions counteracted the patient-specific parameters and possible bias in determining the plaque growth and accumulation (26). Moreover, the blinding of the clinical investigators further ensured an unbiased evaluation of the results.

Concerning the efficacy of the different formulations, there was no significant differences in their action. Despite refraining from mechanical plaque control for 3 weeks, the increases in plaque accumulation, as measured by the Silness & Löe index, were very small and not significantly different between the two types of rinses. There was a statistically significant increase detected within the CHLOREL® (non-alcoholic) rinse group, but it is not clinically significant. This is furthermore supported by the insignificant increase in the gingival index. An important factor that is also presented is the percentage of surfaces that remained plaque free, especially as the individuals partaking in the study had a high level of plaque control before beginning with the oral rinsing. Here too the equivalency of action between the two formulations is clear. But, again within the CHLOREL® group, this difference in plaque accumulation, between initial and final measurements, was found to be significant. These small, from a clinical standpoint but significant statistically, differences in the plaque accumulations could possibly be result of the absence of alcohol in the formulation of CHLOREL®. Ethanol itself does indeed have an antimicrobial effect, albeit small, that may explain this finding (27).

For their anti-inflammatory effect, no difference was discovered either between or within the two solutions. The results for both plaque and inflammation levels are comparable to those shown in other similar recent studies (17, 28).

Table 1. **Initial and final mean values (MV) and standard deviations (SD) of the tested indices/clinical parameters of the study for both solutions***

	Index/Clinical parameter							
	PI		GI		DI		% of sites free of plaque	
Solution	Initial MV (SD)	Final MV (SD)	Initial MV (SD)	Final MV (SD)	Initial MV (SD)	Final MV (SD)	Initial MV (SD)	Final MV (SD)
CORSODYL® (N=10)	0.55 (0.23)	0.69 (0.23)	0.64 (0.32)	0.73 (0.11)	0.0 (0)[a]	0.20 (0.30)[a]	52.55 (19.50)	36.95 (18.17)
CHLOREL® (N=10)	0.52 (0.15)[a]	0.75 (0.19)[a]	0.61 (0.24)	0.77 (0.33)	0.0 (0)[a]	0.06 (0.06)[a]	51.28 (11.82)[a]	32.62 (16.80)[a]

*Same letters in each row indicate statistically significant difference per index, between the initial and final values, for each solution, Wilcoxon signed-rank test, $P < 0.05$.

The DI index, proposed by Tilless and Co-workers (16), was chosen as it focuses, as previously mentioned, on both the amount and intensity. This index allowed a more precise determination of the overall effect of the staining. Tooth staining remains probably the most significant side effect impacting on patient compliance for using CHX products (18, 29). Concerning the level and amount of staining, both rinses were found to significantly increase the staining of the anterior teeth. These teeth were chosen to be examined, as they are most visible and more readily present the aesthetically displeasing major side effect of CHX rinses. Although both formulations increased staining and no strong statistical difference between the two was found, the analysis did suggest an indicative significance of more staining with CORSODYL®. Meaning in larger population samples, this formulation could consolidate the difference seen. This could also most probably be associated to the tendency for a greater impact on the plaque growth that this formulation showed. Not to be overlooked, there is a belief that reductions in tooth colorations are correlated with a diminished clinical activity (30, 31). Thus, this would seem to point to the need of a balance (that must be determined) between the beneficial and sought after antiplaque effect and the unwanted discoloration. Alternatively, antidiscoloration additives may facilitate the reduction in this important side effect without impacting on efficacy (17).

Conclusions

No statistically significant difference in any tested parameter was observed between the two antiseptic solutions. The non-alcoholic chlorhexidine rinse (CHLOREL®) had comparable levels of action as the generally recognized gold standard alcoholic rinse (CORSODYL®). The two formulations are equally effective and safe to use.

Clinical relevance

Scientific rationale for the study

Concern over the possible unwanted effects of alcohol in oral rinses has led to a significant increase in the availability and use of alcohol-free rinses. This, however, generates questions on the possible diminishment of effectiveness; especially of the gold standard CHX rinse.

Principal findings

The present study found that an alcohol-free 0.2% CHX mouthrinse had very comparable clinical effectiveness on *de novo* plaque growth and gingival inflammation, in the absence of mechanical plaque control.

Practical implications

The present findings mean that clinicians can prescribe such a rinse with confidence concerning its efficacy, in the indicated cases.

Source of funding and conflict of interest statement

The authors designed, performed and analysed the clinical study independently from Intermed S.A (Greece), which provided the study products. All authors declare that they have no conflict of interests.

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