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ORIGINAL ARTICLE

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# A preliminary comparison of the effect of 0.3% versus 0.2% chlorhexidine mouth rinse on *de novo* plaque formation: a monocentre randomized double-blind crossover trial

**Abstract:** *Objective:* Chlorhexidine (CHX) is considered the gold standard against gram-negative microorganisms. Little has been written about the simultaneous influence that both time and concentration could have on antiplaque formation effectiveness of CHX. The aim of this study is to compare the clinical and microbiological effectiveness of two different CHX concentrations and time applications in a 4-day plaque regrowth study model. *Material and methods:* Twenty volunteers were enrolled in a randomized double-blind crossover study comparing the effectiveness of CHX 0.3% and CHX 0.2% mouth rinses applied for 15 and 30 s, respectively. Plaque index (PII), total bacterial counts and the detection of specific periopathogens were measured at the 5th day of each mouth rinse application. Taste acceptance was evaluated using a questionnaire. *Results:* Chlorhexidine 0.3% resulted in a statistically greater reductions (10%) in PII and periopathogens compared to CHX 0.2%. Furthermore, patients reported comparable taste acceptance in both groups. *Conclusion:* Chlorhexidine is an effective oral antiseptic. The CHX 0.3% mouth rinse formulation used for 15 s resulted in superior clinical and microbiological outcomes compared to the CHX 0.2% formulation, used for 30 s.

**Key words:** chlorhexidine; gingivitis; oral antiseptics; plaque formation

## Introduction

The cause–effect relationship between dental plaque and periodontal inflammation has been proved long ago and recently reconfirmed (1, 2). On this regard, self-performed oral hygiene, based mainly on toothbrushing and flossing, is essential in maintaining oral health. Despite the importance that oral hygiene instructions have taken in the everyday dental practice, gingivitis is still highly prevalent (3, 4). Strong explanations for this are insufficient patient compliance and improper plaque control (5). To improve plaque control, different antiplaque and antigingivitis dentifrices and mouth rinses are available. It has been demonstrated that their adjunctive use provides better clinical results (6–8).

Previously published studies strongly support the antiplaque and antigingivitis effectiveness of CHX in mouthwash formulation (9). Plaque inhibiting effect of chlorhexidine containing mouth rinses is dose depen-

dent (10), and time length of mouth rinsing reported in clinical trials varies from 30 s up to 1 min. Taste acceptance and rinsing time may be important factors related to patient compliance, therefore in self-performed plaque control.

Little information can be found on the impact that different concentrations and single rinsing time extension could have on the level of CHX mouth rinse effectiveness. Subsequently, the aim of this present study is to evaluate the antiplaque effect of a higher concentration of CHX mouth rinse with shorter rinsing time and the related patient compliance.

## Materials and methods

### Patient selection

Twenty dental volunteer students from Sapienza University of Rome, Italy, 19–25 years old (mean age 21.9) were included in the present monocentre randomized double-blind crossover study. Inclusion criteria were as follows:

- Presence of at least 20 natural teeth (minimum five teeth per quadrant);
- Absence of oral lesions;
- Absence of probing depth $\geq$ 5 mm;
- Absence of removable prostheses or orthodontic bands or appliances;
- Absence of allergy to chlorhexidine;
- Good general health;
- No intake of any medical drug that could influence the outcome of the study.

Female pregnant patients were excluded.

### Ethical approval

The protocol of this study was approved by the Ethical Committee of the 'Sapienza, University of Rome', and all patients received oral and written information on the purpose of the study. All subjects signed an informed consent before the start of the trial.

### Subject allocation

The statistical data collection centre randomly allocated the subjects to one of the treatment sequences by a computer-generated system. Allocation to one of two treatment sequences was enclosed in a sealed envelope, which was opened by the subject, just before the delivery of the antimicrobial treatment regimen.

Subjects were randomly allocated in a first sequence group (Group A) in which mouth rinsing consisted initially in the use of CHX 0.3% for 15 s every 12 h for 4 days, and after a washout period of 10 days, continuing with the CHX 0.2% mouth rinse for 30 s every 12 h for 4 days.

The second sequence group (Group B), on the other hand, used in the beginning the CHX 0.2% mouthwash for 30 s every 12 h for 4 days, followed by the use of CHX 0.3% for 15 s every 12 h for 4 days after a washout period of 10 days.

The two different mouth rinse formulations were provided within identical coded bottles with instructions on rinsing time inserted in the package, to keep the examiner and the subjects blinded. Patients followed the instructions without being informed of the differences between the two products.

### Treatment protocol

Two weeks prior to baseline, all subjects received professional dental hygiene followed by proper instructions and motivation to achieve healthy gingival tissues prior to study beginning. At baseline, after erythrosine disclosure, all subjects received scaling and polishing to remove plaque, calculus and extrinsic stain. Immediately after, they rinsed with the randomly assigned mouth rinse following instructions. Patients were told to avoid rinsing, eating and drinking for an hour after mouth rinsing and to refrain from all other oral hygiene measures including the use of any other mouth rinse, chewing gum or toothpaste for a period of 4 days.

On day 5, all subjects were examined and were asked to report any side effects or adverse event. Subsequently, removal of the present plaque and stains was performed. After a washout period of 10 days during which subjects performed the instructed oral hygiene, the CHX mouth rinse sequence was continued as described above.

### Clinical and microbiological measurements

The evaluation of the effect of CHX on plaque formation was performed clinically (PlI) and microbiologically. The third molars, when present, were excluded from the measurements.

The clinical and microbiological parameters were measured at day 5 and at day 20 (end of study) of each CHX mouth rinse sequence. Microbiologic samples were taken prior to plaque index measurement, from one single tooth for each quadrant using sterile paper points. Real-time PCR was performed for the evaluation of the total bacterial count and for the detection of the following specific periopathogens: *Prevotella intermedia* (Pi), *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* strain 652 (Aa 652), *Aggregatibacter actinomycetemcomitans* strain Jp2 (Aa Jp2) and *Tannerella forsythia* (Tf). After plaque disclosing with erythrosine, plaque index was measured at six sites per tooth for the whole dentition, using the Turesky modification of the Quigley and Hein plaque index (11).

At the end of the study, a 'taste acceptance' questionnaire was given to all subjects to evaluate their attitudes and preferences regarding both products used. In each sequence group, the compliance in using the mouth rinse was evaluated at day 5 and 20 by weighing the amount of mouth rinse remained in the bottle.

### Statistical analysis

The data have been analysed using parametric factorial analysis of variance (ANOVA) considering cohort as grouping factor (between subjects factor, two levels) and dose as repeated

measure factor (within subjects factor, two levels). Plaque index (PII) and log-transformed total bacterial count (BL) were the dependent variables. Their correlation was evaluated by measuring the Pearson correlation coefficient. In addition, a nonparametric analysis (Wilcoxon signed rank test) was carried out for PII comparing the two doses irrespective of the cohort. Level of significance was set at $P$ values lower than 0.05.

## Results

All patients completed the study. No adverse events related to the treatments applied were reported by any of the participants, at any observation time. Frequency distributions of the analysed parameters show rather normal profile of the data, plaque index and total bacteria count (Figs 1 and 2). Plaque index (PII) and total bacterial count (log-transformed) were considered independent, because a linear regression analysis carried out on the whole data set ($n$ = 40) on the hypothesis that BL would predict PII did not show any significant association between the two variables (Pearson correlation coefficient = 0.13; $P$ = 0.41). For both variables, variance was homogeneous across the subgroups. Wilcoxon signed rank test confirmed the parametric analysis.

### PII measurements

There was no main effect of cohort ($F$1, 18 = 0.33; $P$ = 0.57), nor any interaction effect with dose ($F$1, 18 = 1.60; $P$ = 0.22) (Fig. 3). ANOVA for repeated measures indicated that mouth rinsing with 0.3% CHX used for 15 s gave statistically greater reduction in plaque formation compared to 0.2% CHX used for 30 s. This difference was confirmed by the nonparametric analysis as well (Wilcoxon test: $z$ = −2.3; $P$ = 0.02).

Overall, the 0.3% CHX mouth rinse resulted in a greater decrease in the PI index than CHX 0.2% ($F$1,18 = 5.71; $P$ = 0.03). The difference was statistically significant (mean ± standard error for dose 0.3%: 1.31 ± 0.30; mean ± standard error for dose 0.2%: 1.50 ± 0.33) (Fig. 3).

### Total bacterial count

The analysis revealed no main effect of cohort ($F$1, 18 = 0.35; $P$ = 0.56) (Fig. 4) for this parameter as well. However, a highly significant interaction between dose and cohort was noticed. While in one cohort of subjects, total bacterial count did not differ significantly between the two concentrations, in the other it was noticed a statistically significant decrease (of almost 40%) when mouth rinsing with CHX 0.3% was compared to CHX 0.2% ($F$1, 18 = 18.35; $P$ = 0.0004) (mean ± standard error for dose 0.3%: 5.98 ± 0.77; mean ± standard error for dose 0.2%: 3.84 ± 1.27).

### Specific bacterial presence

The real-time PCR analysis of the presence of the selected periopathogens (*Prevotella intermedia*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* strain 652, *Aggregatibacter actinomycetemcomitans* strain Jp2 and *Tannerella forsythia*) showed

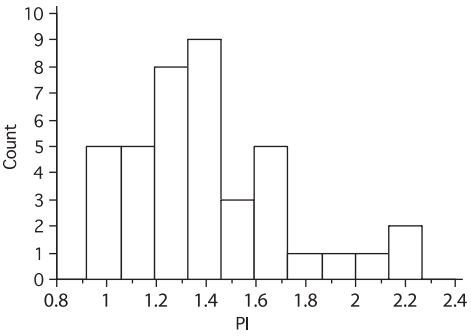


*Fig. 1.* Continuous frequency distribution of the PI index in the analysed sample. Both doses pooled. $n$ = 40.

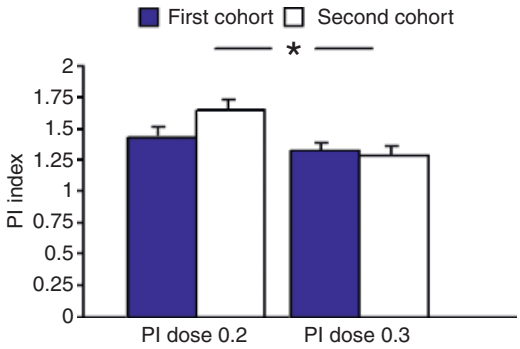


*Fig. 2.* Continuous frequency distribution of the total bacterial count in the analysed sample. Both doses pooled. $n$ = 40.

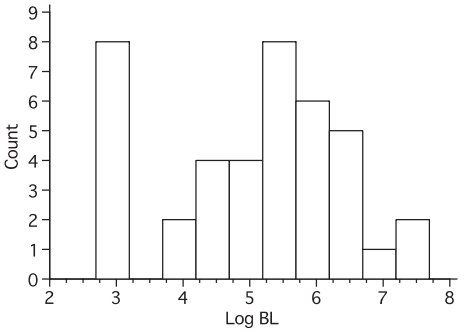


*Fig. 3.* Comparison of the effect of the two different doses on the PI index in the two cohorts of subjects. Values are expressed as means ± standard error. *$P$ < 0.05. See *Results* section for details of the statistical outcome.

statistically significant differences after the use of CHX 0.3% for 15 s if compared to CHX 0.2% used for 30 s (7 with 0.3% versus 12 with 0.2%). Relative data are summarized in Table 1.

### Questionnaire

Among the 20 subjects, 17 selected either 'acceptable' or 'good' as an answer (85%), while only three the 'poor' or 'excellent' alternative of the taste acceptance (15%). Detailed results are presented in Fig. 5.

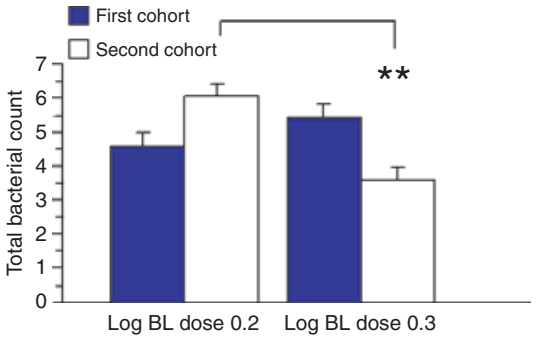


*Fig. 4.* Comparison of the effect of the two different doses on the total bacterial count in the two cohorts of subjects. Values are expressed as means ± standard error. $^{**}P < 0.01$. See *Results* section for details of the statistical outcome.

## Discussion

Mechanical plaque control has become the cornerstone of periodontal therapy, but the ubiquitous prevalence of gingivitis would suggest that control of periodontal biofilm through this mean only is not sufficient (5), especially when mechanical oral hygiene is difficult, compromised or impossible (11). Chlorhexidine is an effective antiplaque and antigingivitis agent that has been safely used as adjunctive gingivitis treatment for years now (12). Furthermore, it is significantly more efficient than triclosan or fluoride toothpaste (13), essential oils and amine fluoride/stannous fluoride mouth rinses (11). The evidence to support these properties of CHX are provided by short-term trials ranging from 4 days to 2 months and long-term clinical trials (mainly of a 6 month follow-up). The 4-day trials are used mainly to evaluate the antiplaque effect of these agents (11). The present study, a short-term trial, reconfirmed the CHX antiplaque properties and its lack of toxicity (14). No adverse effect was reported during the entire study although the short-term follow-up of mouth rinsing could be considered a limit of this protocol regarding the side effects of CHX 0.3%. In this regard, future long-term studies on this higher concentration of CHX could be suggested are needed, to obtain the necessary evidence.

Table 1. **The proportions of specific periopathogens following each chlorhexidine concentration**

| | Periopathogen | | | | |
|---|---|---|---|---|---|
| | Pi | Pg | Aa 652 | Aa Jp2 | Tf |
| 0.2% CHX | 5 | 1 | 1 | 0 | 5 |
| 0.3% CHX | 3 | 0 | 0 | 0 | 4 |

Aa 652, *Aggregatibacter actinomycetemcomitans* strain 652; Aa Jp2, *Aggregatibactr actinomycetemcomitans* strain Jp2; CHX, chlorhexidine; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*; Tf, *Tannerella forsythia*.

(a)

| Score | VAS 0,2 | VAS 0,3 |
|---|---|---|
| P | 3 | 3 |
| A | 12 | 8 |
| G | 5 | 9 |
| E | 0 | 0 |

(b)

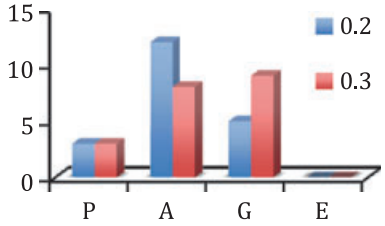


*Fig. 5.* Response of patients regarding the acceptance of taste (score P = poor, A = average, G = good and E = excellent).

Plaque formation was evaluated macroscopically by plaque index calculation and microscopically measuring the total bacterial count and the presence of specific periopathogens. Although neither dental plaque (15) nor periopathogens presence alone (16) are always related to the presence of inflammation and periodontal tissues breakdown, they are important parameters in monitoring professional and self-performed plaque control. Although 4-day plaque regrowth model could be considered not appropriate for the detection of the periopathogenes, which appear in a later stage of biofilm maturation, in the present study, it was possible to detect the presence of some of these pathogens even in the early stages of plaque formation. It was an interesting finding evaluating the influence of CHX on these bacteria, but future investigations are needed. Microbiological studies are being carried out on the role of *Aggregatibacter actinomycetemcomitans* in periodontal tissue destruction hypothesizing that this species function as promoters of human cell receptors for other periopathogens. In the light of this hypothesis, CHX 0.3% gave better results than CHX 0.2%.

The effectiveness of this agent is dose dependent (10). In a recent review on CHX effectiveness (17), it is reported a small but significant difference in favour of plaque inhibition from CHX 0.2% comparing to CHX 0.12%. Our study reported statistically better results of CHX 0.3% compared to CHX 0.2% regarding plaque index and microbiological analysis as well, although being preliminary results only. Further studies and a cost/benefit evaluation are suggested to finally include the use of CHX 0.3% mouth rinse in the everyday treatment protocol.

CHX side effects are mainly local, including (i) taste disturbance, mainly salt taste perception; (ii) desquamative lesions and soreness of the oral mucosa; (iii) parotid swelling; (iv) yellowish/brown staining of the teeth, dorsum of the tongue and acrylic dentures; and (v) in long-term application, stimulated thickening of the pellicle, consequently supragingival calculus formation (18). The increasing of the dose is expected to increase the effectiveness but the side effects as well. Desqua-

mative soft tissue lesions have been reported with the use of drug concentrations exceeding 0.2% or after prolonged application (18). In the present study, within the limits of short-term application of the mouth rinses, no side effects related to the treatment were noticed, including taste disturbance. Taste disturbance should not be underestimated as interfering with the level of patient compliance. Surprisingly, the number of patients choosing the highest given score (Good) of taste acceptance was higher for CHX 0.3% than for CHX 0.2%.

Compliance on the other hand is strongly related to the time a patient spends for the use of this product. Within the oral cavity, CHX is rapidly adsorbed by all surfaces with prolonged bacteriostatic action for up to 12 h (19). Bonesvoll *et al.* (20) and 1977 showed that mouth rinsing with chlorhexidine for 15 s permits up to 50% absorption of the molecule, whereas 30 s increases the uptake by another 25%. Therefore, the reduction in the time extension may not necessarily impair the effect of chlorhexidine on plaque formation or reduce its bactericidal effect against periopathogens. In the present study, it was demonstrated that using a higher concentration of CHX (0.3% versus 0.2%) but in a shorter time of rinsing (15 s versus 30 s), better clinical and microbiological effects were reported, without compromising taste acceptance. Aware of the limits that the small sample size presents, further randomized controlled studies are needed for the evaluation of these results and the possible side effects related to a prolonged use of this higher CHX concentration mouth rinse.

## Clinical relevance

Chlorhexidine (CHX) 0.3% mouth rinse applied for 15 s could be suggested as adjunctive treatment of periodontal diseases, based on significantly better results than CHX 0.2% used for 30 s in controlling macroscopically and microscopically plaque formation. Furthermore, the combination of an increased concentration and a shorter application time gave better results with a positive influence on patient compliance. The new proposed protocol of CHX mouth rinse concentration and time extension could facilitate the self-performed oral hygiene measurements and the maintenance of healthy gingival tissues.

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## Conflict of interest

Authors declare no conflict of interest.

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