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Evaluation of chlorhexidine 0.05% with the adjunct of fluoride 0.05% in the inhibition of plaque formation: a double blind, crossover, plaque regrowth study

Abstract: *Objective:* The aim of this study was to evaluate the effect of mouthrinses containing 0.05% chlorhexidine + 0.05% fluoride solution on early dental plaque regrowth. *Materials and methods:* Thirty periodontally healthy subjects were included in the study. A crossover 4-day plaque regrowth protocol was adopted. The test product was initially used in 15 patients, while a placebo was administered to the other 15 patients. Then, after a washout period, each patient used the other product. No other oral hygiene manoeuvre was allowed. Full-mouth plaque and bleeding scores (FMPS and FMBS) were evaluated at baseline and after 4 days. *Results:* All subjects completed the study. The mean age was 27 ± 8.4 years. Five patients were smokers with a mean daily consumption of 1 ± 2.5 cigarettes. FMPS at baseline was 8.0 ± 4.4 for control group and 7.9 ± 3.8 for test group, without significant difference. After the 4-day plaque regrowth the mean FMPS significantly increased to 31.9 ± 16.5 and 36.3 ± 16.1 for control and test group, respectively (no significant difference between the two groups). *Conclusions:* The test product was safe and well tolerated by subjects. The similar outcomes of the two experimental groups suggest that the two products have an equivalent effect on early dental plaque regrowth. Studies with longer follow-up are needed to clarify whether there is a beneficial long-term effect of daily rinses with the tested solution.

Key words: bacterial plaque; chlorhexidine; fluoride; gingival inflammation

Introduction

The control of plaque accumulation on teeth surfaces is fundamental for the prevention of oral pathologies strictly correlated to the presence of bacterial biofilm, such as caries and periodontal diseases (1). Oral hygiene manoeuvres, if well performed through the use of toothbrushes and interdental devices, are useful in reducing significantly dental plaque accumulation and related diseases also in long term (2–4).

Though, it was demonstrated that, also after proper oral hygiene manoeuvres, plaque removal is far to be complete, being strictly dependent on tooth position, patients' dexterity, as well as tooth anatomy (5–8).

The need to improving the control of plaque accumulation has led to the development of a number of antimicrobial agents as essential oils (9, 10), cetylpyridinium chloride (11) and chlorhexidine (12). Fluoride molecules were also demonstrated to be effective *in vitro* in reducing bacterial biofilm formation (13).

Chlorhexidine (CHX) is one of the most used oral antimicrobial agents for which different formulations are available. Chlorhexidine shows good substantivity, and, at high concentrations (0.2% or more), it is bactericidal, causing a lethal damage to the bacterial membrane. ICHX is active towards both gram-negative and gram-positive bacteria and yeast as well (14). At low concentrations, chlorhexidine can reduce the bacterial metabolism through different pathways (14).

Chlorhexidine has been studied as an antimicrobial agent in the treatment for gingivitis since 1970 (15). A recent systematic review of the literature has shown that both 0.12 and 0.2% CHX mouthwashes were useful in the reduction of both plaque and gingival index (12). Furthermore, only a small advantage in using 0.2% concentration was demonstrated (12).

Some studies evaluated the use of low concentrations (0.05%) of CHX as an adjunct to daily oral hygiene for the control of plaque formation (16–19).

Two studies demonstrated that rinses with 0.05% CHX with the adjunct of 0.05% cetyl-pyridinium chloride were effective in reducing plaque and gingivitis and in decreasing the microbial load in the short and medium term (18, 19).

The aim of the present comparative study was to evaluate, through a plaque regrowth protocol, the effect of rinses with 0.05% CHX + 0.05% fluoride solution in reducing plaque formation and signs of gingivitis.

Study population and methodology

The study protocol was approved by the Research Committee of the Centre for Research in Oral Health of the University of Milan. This study was conducted following the principles embodied in the Helsinki Declaration of 1975 for biomedical research involving human subjects as revised in 2000 (20). All patients were informed about the study protocol and signed an informed consent form before beginning the study.

Sample size and randomization

Patients were selected from those attending the Dental Clinic of the IRCCS Istituto Ortopedico Galeazzi in Milan.

Inclusion criteria for the study were the following:

- Adult patients, older than 18 years of age;
- Absence of systemic diseases;
- No chronic medication intake;
- At least 20 teeth without caries.

Exclusion criteria were as follows:

- Pregnancy or lactation;
- Antibiotic treatment within 6 months before beginning the study;
- Topical antimicrobial treatment within 4 weeks before beginning the study;
- Periodontitis (more than three sites with probing depth $\geq$ 3 mm);
- Presence of active infection (for example periodontal or endodontic abscess).

A total of 30 patients meeting the inclusion criteria were recruited for the study.

Through computer-aided randomization, the subjects were divided in two groups of the same size named group A and group B. Several parameters were balanced by computer between two groups:

- Patient's age;
- Patient's gender;
- Presence/absence of diabetes mellitus;
- Smoking (number of cigarettes a day);
- Full-mouth bleeding score (FMBS%) and full-mouth plaque score (FMPS%) measured at baseline evaluation.

A 4-day plaque regrowth study, as described by Addy in 1983 (21), with a crossover design, was used.

Treatment protocols and timing

In Fig. 1, timeline of the study is represented. Soon after baseline examination, clinical parameters were evaluated and recorded. Then, in the same visit, professional oral hygiene was performed and oral hygiene instructions were given with the aim of standardizing oral hygiene manoeuvres. After 2 weeks without treatment, patients were recalled and clinical parameters were recorded. Then, the selected product was given to subjects, along with the usage instructions. Patients were instructed to rinse their mouth with 10 ml of the given product for 1 min twice a day for 4 days avoiding any other oral hygiene manoeuvre. After this period, clinical parameters were recorded and then a washout period of 2 weeks followed. Then, the protocol continued inverting the products according to a crossover design and with the same timing and mode of use previously described. In this way, each subject received both treatments sequentially.

The test product was chlorhexidine 0.05% mixed with 0.05% fluoride solution (CuraseptTM; Curaden Healthcare Srl,

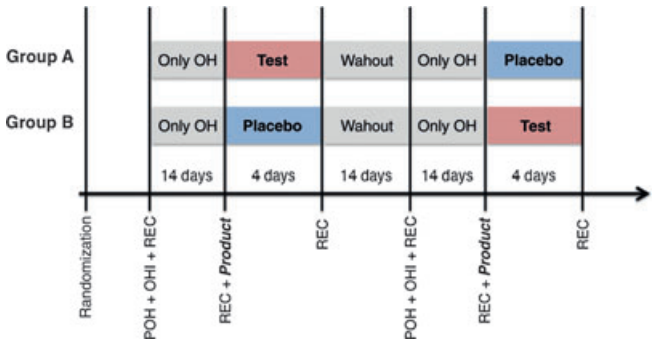

Fig. 1. Diagram of study timeline.

Saronno, Italy) with antidiscoloration system (ADS®; Curaden Healthcare Srl).

Parameters evaluation

The following parameters were evaluated at each follow-up visit and recorded in a specific form:

- Full-mouth plaque score% (FMPS%) representing the percentage of teeth surfaces with plaque accumulation evaluated using a periodontal probe: the probe was used on each tooth surfaces for every teeth and then the percentage of positive surfaces was calculated;
- Full-mouth bleeding score% (FMBS%) representing the percentage of sites with bleeding on probing;
- Presence of pigmentation on teeth surfaces;
- Occurrence of complications.

Statistical analysis

Student's *t*-test was used to compare FMPS% and FMBS% changes over time. A significance of $P < 0.05$ was considered as the threshold level for refusing the null hypothesis that both products were equally useful in plaque control.

Results

A total of 30 patients were included in the study. No dropout was recorded. Baseline subject characteristics are listed in Table 1. No significant difference existed for age, gender, smoking habits and alcohol consumption between the two groups.

FMPS% and FMBS% at different follow-up visits are shown in Table 2.

FMPS% at baseline was rather low for both groups, averaging 8.0 ± 4.4 for placebo group and 7.9 ± 3.8 for test group (no significant difference between groups). The increase in plaque levels during the 4-day time frame was significant in both groups ($P < 0.0001$). The mean FMPS was 31.9 ± 16.5 and 36.3 ± 16.1 for placebo and test group, respectively (no significant difference between groups). On the average, FMPS% increased more than four times, with great variability among subjects.

Pretreatment FMBS% was low for either groups. A statistically significant increase was found at the 4-day examination, with no significant difference between the two groups. In this short period, none of the patients experienced gingivitis, and mean FMBS% did not exceed 5% (4.0 ± 3.3 for placebo group and 4.5 ± 2.1 for test group).

None of the patients referred complications during or after the treatment phases. No signs of staining were detected in both groups.

Patients declared to appreciate the taste of both products with a slight preference for the test one.

Table 1. **Baseline sample characteristic**

Characteristic	Group A *n* = 15	Group B *n* = 15	Total *n* = 30	Difference
Gender (M/F)	10/5	9/6	19/11	NS
Age (Mean ± SD) (years)	27 ± 8.1	27 ± 8.8	27 ± 8.4	NS
Diabetes (*n*)	1	0	1	NS
Smoking (Mean *n* cigarettes/day)	1.2 ± 3.1	0.7 ± 1.7	1 ± 2.5	NS
Smokers (*n*)	2	3	5	NS
Alcohol consumption (Mean *n* glasses)	0.2 ± 0.6	0.3 ± 0.6	0.2 ± 0.6	NS
FMPS%	9.7 ± 5.7	7.7 ± 4.4	8.7 ± 5.1	NS
FMBS%	3.0 ± 2.2	2.3 ± 1.7	2.7 ± 2.0	NS

FMBS, full-mouth bleeding scores; FMPS, full-mouth plaque scores; NS, not significant.

Discussion

The 4-day plaque regrowth study is one of the most used models to study oral antiseptics (22, 23). It was first implemented by Addy and coworkers in 1983 (21). It was mainly aimed to study the plaque inhibitory effect of a formulation *in vivo* while any oral hygiene is stopped during the test phase. Generally for such model, a crossover approach is used, as in the present study (22).

This kind of study is particularly indicated to evaluate the capacity of the product itself to inhibit plaque neoformation, but in the literature it was usually used with concentrations of CHX higher than those used in the present study.

In 2005, a study was published which compared a 0.2% CHX solution with 0.12% CHX with the adjunct of cetylpyridinium chloride (CPC) with a 3-day plaque accumulation model (24). The authors concluded that both tested products were comparable in terms of clinical results (24).

A study by Stoeken and coworkers, published in 2007, compared three different CHX formulations in a 3-day 'de novo' plaque formation model (25). A 0.12% CHX spray was compared with 0.2% CHX spray and 0.2% CHX mouthwashes. It was concluded that mouthwashes were more effective in inhibiting plaque regrowth and that it could be due to the capacity of reaching all sites in the mouth (25).

In 2010, the same study design was used to compare a 0.12% CHX dentifrice gel versus tray application of 1% CHX gel, showing that higher concentrations of CHX resulted in higher effect in inhibition of plaque accumulation (26).

Chlorhexidine was also compared to other products, using the same study design.

Pizzo *et al.* (27) compared different antimicrobial formulations (0.2% CHX, 0.12% CHX, 0.05% CPC and 0.03% triclosan) both as sprays and as mouthrinses using a 4-day plaque regrowth model. The authors confirmed that CHX-based formulations were more effective than others and that rinses were superior to sprays (27).

In 2008, Paraskevas and coworkers published the results of a 3-day plaque regrowth study which compared 0.2% CHX

Table 2. **Plaque and bleeding indexes**

	Baseline (T0)		Before treatment (T1)		After treatment (T2)		Diff (T2–T1)	
	Test	Control	Test	Control	Test	Control	Test	Control
FMPS%	9.4 ± 5.2	9.7 ± 6.7	7.9 ± 3.8	8.0 ± 4.4	36.3 ± 16.1	31.9 ± 16.5	28.4 ± 15.0	23.9 ± 14.8
FMBS%	2.6 ± 2.5	2.1 ± 1.9	2.6 ± 1.9	2.2 ± 2.0	4.5 ± 2.1	4.0 ± 3.3	1.9 ± 2.4	1.8 ± 3.0

FMBS, full-mouth bleeding scores; FMPS, full-mouth plaque scores.

rinses to a chlorine dioxide mouthrinse, demonstrating that the test product (chlorine dioxide) was a less potent plaque inhibitor than CHX (28).

The present study compared low concentration of CHX (0.05%) with the adjunct of fluoride (0.05%) versus a placebo.

No differences could be evaluated in terms of plaque accumulation between the two products neither in terms of gingival inflammation. Plaque regrowth during the test phase was not statistically correlated with the increase in gingival inflammation (evaluated trough the FMBS%). The tested product did not produce any adverse effect in patients and was generally appreciated in taste by subjects.

These results differed from the ones in scientific literature, although no investigators before tested this precise formulation.

In 2004, Santos *et al.* (18) evaluated the clinical and microbiological activity of a new mouthrinse formulation for patients in supportive periodontal care. In that study, a test product containing 0.05% CHX + 0.05% CPC was compared with a placebo evaluating clinical parameters after 15 days. Only plaque accumulation index was significantly lower in test group together with total bacterial count.

A further report by Escribano *et al.* (19) evaluated the same product of the previous article by Santos versus placebo. The test phase was modified, and patients rinsed with the product, together with oral hygiene manoeuvres, for 3 months. The tested product was found to be useful in reducing plaque and gingivitis during supportive periodontal care.

Considering the results of the present study, these could be considered as preliminary, and we cannot exclude that the same tested product could have a positive effect in medium or long term, according to the indications provided by the manufacturer and the scientific literature.

Further medium- or long-term studies will be useful in deeply evaluating the effect of a low concentration of CHX mouthwash with the adjunct of fluoride in reducing plaque accumulation.

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