Comparison of commercially available 0.2% chlorhexidine mouthwash with and without Anti-Discoloration System. A blinded, cross-over clinical trial

Erik Klepsland Mauland, Hans R Preus and Anne Merete Aass

Department of Periodontology, Institute of Clinical Odontology, Faculty of Dentistry, University of Oslo, Norway.

Running title: Chlorhexidine with and without ADS

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Corresponding author: Erik Klepsland Mauland, Department of Periodontology, Institute of Clinical Odontology, Faculty of Dentistry, POB 1109 Blindern, Oslo, Norway

Tel: +47 228 52 060; Email: e.k.mauland@odont.uio.no

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Abstract:

Aim: To evaluate clinical performance and side-effects of two commercially available 0.2% chlorhexidine mouthwashes after periodontal surgery, one with (test) and one without (control) an anti-discoloration system.

Material and methods: This single-centre, cross-over clinical trial included 38 patients undergoing two sessions of periodontal flap surgery. The participants used two different 0.2% chlorhexidine products, one with and one without an anti-discolouration system, in the 14-day post-operative periods. Plaque, gingival inflammation, tooth staining, side-effects and patient preference were evaluated.

Results: The control mouthwash (without an anti-discolouring system) produced significantly lower plaque - ($p=0.02$) and gingival index ($p=0.01$) compared to the test mouthwash. The test mouthwash produced significantly less staining in the gingival ($p=0.002$) and approximal areas ($p=0.0004$), but no difference was detected in the buccal area of the teeth. The patients did not show preference for any of the mouthwashes.

Conclusion: Chlorhexidine mouthwash without an anti-discoloration system resulted in significantly lowered plaque- and gingival index compared to chlorhexidine mouthwash with an anti-discoloration following periodontal surgery. No difference in patient preference was found despite less side effects produced by the chlorhexidine mouthwash with the anti-discolouration system.

Clinical relevance:

Scientific rationale for the study*:* Chlorhexidine (CHX) is used as compensation for abstained tooth brushing after periodontal surgery. The main side effect of CHX is tooth staining, and an Anti-Discoloration System (ADS) has been added to CHX mouthwashes to reduce this (CHX+ADS). **Principal findings***:* CHX+ADS provided inferior plaque-inhibition but less tooth discoloration than the CHX only mouthwash. **Practical implications:** CHX proved better plaque prevention than CHX+ADS. Although discoloration was more pronounced in the CHX group, the patients reported no preference for either, suggesting the CHX product without ADS to be preferred.

Introduction:

Chlorhexidine (CHX) is a frequently used biocide with a well-documented dental plaque-inhibiting effect (Gjermo, Rølla, & Årskaug, 1973; Löe & Schiøtt, 1970). The CHX concentrations of commercially available mouthwashes in Norway range between 0.05% and 0.20%, and a recent systematic review (Berchier, Slot, & Van der Weijden, 2010) and a clinical study (Haydari et al., 2017) showed that 0.20% CHX exhibited significantly better plaque-inhibiting effect than 0.12% and 0.06%, respectively. Moreover, additives, to enhance taste, colour, and consistency have been suggested to alter the antimicrobial capacity (Herrera et al., 2003).

Although minor taste alterations and mucosal discomfort have been reported (Cortellini et al., 2008; Haydari et al., 2017), the most common side effect of CHX mouthwash is staining of teeth, which has been suggested to reduce patient compliance (Cortellini et al., 2008; Ellingsen, Rølla, & Eriksen, 1982; Eriksen, Nordbø, Kantanen, & Ellingsen, 1985; Gjermo et al., 1973). In an effort to increase the latter, an Anti-Discoloration System (ADS) has been added to a CHX recipe. Reports on clinical performance of the CHX+ADS combination suggest similar plaque-inhibitory capacity and less tooth staining (Bernardi, Pincelli, Carloni, Gatto, & Montebugnoli, 2004; Cortellini et al., 2008; Solis, Santos, Nart, & Violant, 2011). These studies compared the mouthwashes while study subjects performed tooth brushing (Cortellini et al., 2008, Bernardi et al., 2004; Solis et al., 2011). The hypothesis of the present study was that the mouthwash without and with ADS showed similar plaque and gingivitis-inhibiting effects with less tooth staining from the latter. Therefore, the aim of the present study was to compare clinical performance as well as side effects, of two commercially available 0.2% CHX

mouthwashes, one with and one without ADS, after periodontal surgery when the patients had to abstain from mechanical tooth cleaning in the areas of therapy.

Material and Methods:

This single-centre, blinded, cross-over clinical trial comparing an ADS containing 0.2% CHX mouthwash (test) to a 0.2% CHX mouthwash without an ADS (control) was performed at the Department of Periodontology, University of Oslo, from March to December 2019. The test product (Curasept®, Curaden GMBH, Switzerland) and the control product (Corsodyl®, GlaxoSmithKline, Brentford, UK) are commercially available ethanol-free CHX mouthwashes.

***Study population*:**

Sample size: It was calculated that 30 participants were needed to detect a difference for the primary outcome variable Plaque Index (PlI) of 0.25 between the groups with 80% power and a 5% confidence interval. The enrolment continued until 38 patients were included to compensate for potential 20% drop out during the study period.

Thirty-eight patients, scheduled for periodontal surgery in at least two sextants, being in otherwise good general health, and classified as periodontitis stage III or stage IV (Tonetti, Greenwell, & Kornman, 2018) volunteered for the study. Smokers, non-smokers and patients with diabetes participated in the study (Table 1). The patients were supplied with the mouthwashes, but received no other financial compensation. Simple, restricted randomization was carried out using a computer-generated random allocation table (Altman, 1991) to decide the order of the two mouthwashes that were used following the two surgeries. A dental assistant enrolled the patients and allocated the study subjects to test or control groups. The main investigator was kept blinded until the data collection was completed. Figure 1 shows the flow

of patients through the study. All patients signed an informed consent, and the regional ethical research committee approved the study (REK. 2018/1588). Clinicaltrials.gov ID-number is NCT04223076.

Clinical procedures

Immediately prior to each surgical session, the teeth were polished with a low speed rubber cup and pumice (Pumice, Apotekherproduksjonen AS, Oslo, Norway) to remove any tooth staining at baseline. The patients were treated by flap surgery as indicated, performed by five post-graduate candidates in periodontology.

Following all surgeries, the main investigator (EKM) told each patient to rinse 1 min with 10ml CHX mouthwash BID during the next 14 days, starting the first evening/night following surgery. The patients were instructed to brush teeth as usual in the quadrants not subject to surgery and avoid brushing in the quadrant subject to surgery. After this procedure, they were to rinse with water for 30 sec to avoid possible carryover interactions with toothpaste, and thereafter rinse with the test- or control product as instructed. No mechanical tooth cleaning was performed in the area of surgery until suture removal 14 days post-surgery. A period of at least 14 days was mandatory from the first CHX rinse was completed until the second surgery.

***Clinical recordings*:**

The following parameters were recorded by one examiner (EKM) at each follow up visit: Plaque Index (PlI) (Silness & Löe, 1964), Gingival Index (GI) (Löe & Silness, 1963), tooth staining index (Lobene, 1968), modified by Gründemann (MLSI) (Gründemann, Timmerman, Ijzerman, Velden, & Weijden, 2000) (Figure 2).

Prior to the experiment, the examiner was calibrated by one experienced periodontist (AMA). Intra-examiner agreement for the MLSI was calculated by EKM evaluating 76 teeth in clinical photographs. The photographs were evaluated twice, with one-week interval. Intra-examiner agreement was expressed by weighted Cohen`s kappa (k_w).

A questionnaire with the intent to monitor patient eating and drinking habits, subjective side-effects as well as perception and preference of the two different products during the study was presented to the participants at each post-surgical examination (Table 3 and 5).

***Statistical methods*:**

The analysis of data was performed by descriptive statistical analysis. Percentage distribution, mean and standard deviation (SD), median and interquartile range (IQR) and range are shown. Normality of continuous variables were analyzed on histogram, and by Shapiro-Wilk test. Since the normality assumption was not satisfied, Wilcoxon Signed Rank test was used to compare the median differences of the two different test results. To analyze the change in proportion for two paired data (two treatments) McNemar test was used. Significance limit was set at $p<0.05$. Statistical Package for STATA (Stata version 14.0; College Station, TX, USA) was used for the statistical analyses.

Results:

Thirty-one of 38 patients completed the study (82%). Six patients were lost to follow-up and one patient was excluded due to violation of protocol. Demographic characteristics are shown in Table 1. *Intra-examiner* agreement value (k_w) for the MLSI evaluation was 0.92.

The control group (CHX) showed significantly lower PlI and GI recordings than the test group (CHX+ADS) (Table 2). Different levels of MLSI tooth staining were found between the groups as zone A and zone G were significantly more stained in the control group, whereas no difference for zone I was observed (Figure 2, Table 2).

Self-perceived discoloration of teeth, as reported in the questionnaire, was similar in the CHX+ADS and the CHX group, while significantly more self-perceived discoloration of the tongue was reported in the CHX only group (Table 3). No difference in consumption of strongly coloured foods and drink among the test and the control group was detected during the 14-days experimental period.

Self-reported taste alterations and mucosal discomfort were significantly more frequent in the CHX only group. No difference in altered sensation of the oral cavity was detected (Table 3), and no significant difference in mouthwash preference was observed among the study participants (Table 5).

Nine out of 31 subjects (29%) were smokers, and although they were educated and advised to quit smoking during the pre-treatment phase, none were successful in doing so fully. Smokers showed in general higher MLSI scores than non-smokers, but there was no significant difference in PlI and GI between smokers and non-smokers (Table 4).

Discussion:

In the present study, the plaque preventing capacity was significantly better (PlI=0.4) in the control - (CHX) as compared to the test group (PlI=0.8) (CHX+ADS), which agrees with a study by Arweiler, Boehnke, Sculean, Hellwig, and Auschill (2006), who reported a PlI of 0.4

for a non-ADS CHX mouthwash and PlI=1.0 for the ADS containing mouthwash. However, the results are in contrast to Bernardi et al. (2004) and Solis et al. (2011), possibly because these studies evaluated the mouthwashes in patients brushing their teeth simultaneously.

The finding that the ADS-containing product produced less tooth staining than the CHX control is in agreement with previous studies (Bernardi et al., 2004; Cortellini et al., 2008; Solis et al., 2011). The anti-staining properties of the ADS-containing mouthwash have been investigated by several authors. Addy, Sharif, and Moran (2005) showed in an *in vitro* study that dietary chromogens were able to react with the CHX+ADS mouthwash, and found no difference in staining compared to the control solution without ADS. In the present study no difference in staining was observed in zone I (Figure 2, Table 2), indicating that staining reduction was more effective on the outer borders of the tooth surface, where the mechanical wear from lips, cheeks, tongue and abrasive food particles is limited.

Arweiler et al. (2006) have suggested that components of the ADS system may compete and/or interact with the active CHX-molecules, thereby reducing the plaque inhibitory potential of the mouthwash. An investigation of four different mouthwashes containing 0.12% CHX found anti-microbial action to differ between the formulas, which indicate the potential of additives to alter the plaque-inhibitory properties of the product (Herrera et al., 2003).

No difference in self-perceived tooth staining was reported between the test and the control mouthwash. The registration of tooth staining in zone I was not significantly different between groups, and this zone is probably the area that is the most conspicuous. The participants specified intake of chromogenic foods or drink (y/n) in the questionnaire. Using the test product, 22 subjects reported drinking coffee, 16 reported drinking tea and 14 reported consuming food

of strong color. The corresponding figures when using the control product were 22, 14 and 15, respectively. As expected, due to the cross-over design, the habits of the study subjects were almost identical during the two post-operative periods. It must be noted that the study subjects were not instructed to avoid specific food or drinks. Although the study population is considered representative for periodontitis patients undergoing surgical treatment, one cannot rule out that aesthetic concerns and awareness of side effects may be different in other populations.

Although mechanical tooth cleaning may be re-instituted after a shorter period of time, this study elected a 14-day non-brushing period for the area of surgery, since diabetics and smokers may observe slightly longer time for wound healing (Grossi et al., 1997; Kido et al., 2017).

The smokers of the population (29%) showed – as a group – higher MLSI scores than the non-smokers. The staining properties of tobacco use has been discussed extensively in the literature, as also was confirmed in this study. However, since this was a crossover study, and the test persons confirmed a comparable behaviour throughout the two periods of surgery and follow-up, the results would not be distorted.

The number of lost to follow up was slightly elevated in the present study as compared to Cortellini et al. (2008). Six patients were lost to follow-up and one patient was excluded due to poor compliance. An examination of these participants revealed no different demographic characteristics as compared to the subjects who completed. However, the power of the study was still in line with the sample-size calculations.

Conclusion:

1. The 0.2% CHX+ADS was less effective in preventing plaque and gingivitis.
2. The 0.2% CHX+ADS produced significantly less tooth stain and mucosal discomfort.

3. The patients did not show a preference for any of the two products despite the difference of self-reported side-effects.

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322 Table 1: Demographic characteristics of the study subjects

	n	**%**
Total Sample size	31	100
Male	18	58
Female	13	42
Median age (range)	52 (25 – 74)	
Smokers	9	29
Diabetes	2	6

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Table 2: Descriptive statistics at follow up visits for test and control product (Wilcoxon signed rank test)

N=31	**Test** Mean±SD Median I(QR) Range	**Control** Mean±SD Median I(QR) Range	**P-value**
PLI	0.8±0.5 0.8 (0.4-1.2) 0-1.5	0.4±0.3 0.3 (0.2-0.5) 0.1-1.3	0.0004***
GI	0.8±0.4 0.8 (0.5-1.2) 0-1.6	0.4±0.3 0.4 (0.2-0.7) 0-1.0	0.0002***
MLSI zone I	0.1±0.3 0 (0.0-0.0) 0-1.0	0.2±0.3 0 (0.0-0.0) 0-1.0	0.9374
MLSI zone A	0.1±0.3 0 (0.0-0.1) 0-1.0	0.4±0.5 0.5 (0.0-1.0) 0-1.0	0.0004***
MLSI zone G	0.1±0.2 0 (0.0-0.5) 0-1.0	0.3±0.4 0 (0.0-0.7) 0-1.0	0.0024**

***P<0.001
** P<0.01
* P<0.05

Table 3: Self-reported side effects following use of mouthwash (McNemar test)

Mouthwash	Number of participants	S1	S2	S3	S4	S5
Test	31	6	3*	5	2	0*
Control	31	6	7*	10	2	6*

*p<0.05
S1: Self perceived discoloration teeth
S2: Self perceived discoloration tongue
S3: Taste alterations
S4: Altered sensation
S5: Mucosal discomfort

Table 4: Tooth staining in smokers and non-smokers following mouthwash (McNemar test)

Smoking status	Number of participants	MLSI zone I	MLSI zone A	MLSI zone G
Smoker	9	0.20	0.45*	0.29
Non smoker	22	0.15	0.24*	0.17

*p<0.05

Table 5: Preferred product (PP) among participants (N) (McNemar test)

Mouthwash	N	PP
Test	31	17
Control	31	14

No significant differences

Figure legends.

Figure 1: CONSORT Flowchart of study

Figure 2: Discoloration report figure (Modified from Cortellini et al. (2008))

Flow diagram for crossover trial comparing 0.2% Chlorhexidine with and without an ADS