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ORIGINAL ARTICLE

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Therapeutic efficacy of chlorhexidine-based mouthwashes and its adverse events: Performance-related evaluation of mouthwashes added with Anti-Discoloration System and cetylpyridinium chloride

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Abstract

Objectives: To compare 3 mouthwashes: 0.20% chlorhexidine (CHX) with Anti-Discoloration System (ADS), 0.20% CHX and 0.12% CHX with 0.05% cetylpyridinium chloride (CPC), in terms of reduction of plaque and gingival bleeding and side effects.

Methods: Mild gingivitis patients were randomly divided into three Groups: they underwent professional oral hygiene and received instructions: oral rinse with 10 mL for 1', twice a day, 30' after tooth brushing, for 14 days. Primary outcomes were plaque and gingival bleeding, assessed with Plaque Control Record and Gingival Bleeding Index. Feedback questionnaire and spectrophotometer evaluated secondary outcomes: adverse events. Timing of the study was T0 (baseline), T1 (professional oral hygiene) and T2 (14th day after mouthwash use).

Results: Sixty-six patients were recruited, two patients dropped out, and 64 patients completed the study. PCR T1-T2 mean variation was 30.67 (SD = 15.22; 95% CI 23.55 to 37.80; $P = 0.000$), 19.93 (SD = 11.03; 95% CI 14.90 to 24.95; $P = 0.000$) and 16.24 (SD = 15.35; 95% CI 9.60 to 22.88; $P = 0.000$) respectively in Groups 0.2% CHX + ADS, 0.2% CHX and 0.12% CHX + CPC. GBI mean variation (T0-T2) was −9.82 (SD = 9.27; 95% CI −5.48 to 14.16; $P = 0.000$), −19.31 (SD = 11.33; 95% CI −14.15 to −24.47; $P = 0.000$) and −21.13 (SD = 12.56; 95% CI −15.70 to −26.56; $P = 0.000$) respectively in Groups 0.2% CHX + ADS, 0.2% CHX and 0.12% CHX + CPC. Statistical significance was found in lower efficacy of 0.2% CHX + ADS Group. Patients tolerated 0.12% CHX + CPC mouthwash better in bleeding perception (95.5%; $P = 0.046$), burning sensation (13.6%; $P = 0.006$), and mouthwash taste (100%; $P = 0.000$). Results on staining were no statistically significant ($P = 0.106$).

Conclusions: Addition of CPC allows reduction of CHX percentage in mouthwash formulation while keeping equal efficacy and less side effects. ADS addition decreases CHX efficacy in reducing plaque and bleeding, while resulting more tolerated than CHX.

KEYWORDS
Anti-Discoloration System, cetylpyridinium chloride, chlorhexidine digluconate, mouthwash

1 | INTRODUCTION

The central role of oral biofilm in caries and periodontal diseases has been demonstrated.[1,2] The best prevention strategy is mechanical removal of bacterial plaque through daily oral hygiene procedures and professional oral hygiene.[4,5] Dentists and dental hygienists frequently recommend additional aids, the most widely used is chlorhexidine (CHX).[9,10] Indeed, associated with debridement, CHX represents an effective option for the treatment of plaque-induced gingival diseases, due to inhibition of bacterial biofilm build-up and microbial re-colonization of already treated sites.[10,11] CHX's mechanism of action may cause some reversible side effects such as alteration of food taste, mucosal irritation,[13,14] unsightly yellow-brown pigmentation of enamel, tongue and composite restorations. These unpleasant effects may undermine patients compliance towards treatment.[13,16] Several molecules have been associated with CHX to maximize its antimicrobial efficacy or reduce adverse events. One of this is cetylpyridinium chloride (CPC), an amphiphilic quaternary compound, with a demonstrated efficacy in increasing antimicrobial activity when incorporated into oral hygiene products.[17,18] It is a cationic detergent, whose interaction with cellular membranes results in leakage of cellular components, disruption of bacterial metabolism, inhibition of cells growth, and cells death.[20] Anti-Discoloration System system (ADS) is designed to reduce CHX-induced pigmentation. Based on ascorbic acid and sodium metabisulphite,[16] ADS interferes with the two main pigmentation processes: the protein denaturation leading to metal sulphides formation; and the Maillard reaction, which develops brown staining substances, known as melanoidins.[5,21]

The aim of this study was to compare the efficacy of three mouthwashes (0.2% CHX + ADS, 0.2% CHX and 0.12% CHX + 0.05% CPC), in reducing bacterial plaque and gingival bleeding as well as side effects, in association with oral home care.

We followed the CONSORT guidelines for an accurate reporting of this RCT.[22]

2 | STUDY POPULATION AND METHODOLOGY

2.1 | Trial design

This was a single centre, double-blinded, three parallel-Group study conducted at the First Observation Dental Unit of Oral and Maxillo-Facial Sciences Department, Sapienza University of Rome. The ethical committee of Polyclinic Umberto I approved the study protocol with the resolution n. 2779 of 30/05/2013.

2.2 | Participants

Patients with mild gingivitis were randomly divided into three Groups to receive a digluconate CHX without alcohol mouthwash:

- Group ADS: 0.2% CHX with Anti-Discoloration System (ADS) (Curasept, Curadent Healthcare SpA, Saronno, VA, Italy);
- Group CHX: 0.2% CHX (Dentosan, Recordati SpA, Milano, MI, Italy);
- Group CPC: 0.12% CHX with 0.05% cetylpyridinium chloride (CPC) (Gum Paroex, Sunstar Italiana SRL, Saronno, VA, Italy).

Gingivitis was evaluated with Gingival Index (GI): a dental hygienist established colour, texture and tropism of gingiva using the PCP UNC 15 probe sliding along the gingival sulcus to detect bleeding on probing of each of the four tooth surfaces (mesial, buccal, distal and oral) and then, he assigned, to each of them, a score from 0 to 3. The individual GI was calculated on the second and fifth sextant and on lower first molars, dividing the total score by the number of the examined surfaces.[23]

The GI was assessed at baseline only to recruit patients by gingivitis severity.

Inclusion criteria were as follows: Patients aged 18-40 years, with no systemic disease at the anamnestic questionnaire, presenting a Gingival Index (GI) between 1.1 and 2.0.

Exclusion criteria were as follows:

- All patients aged under 18 and over 40, to have only adults and to reduce the age range;
- Less of 24 teeth;
- Pocket depth ≥5 mm on one or more teeth;
- CAL (clinical attachment level) >2 mm[24];
- Cavities on upper and lower incisors;
- Patients taking daily: coffee (more than three cups), tea (more than two cups), red vine (more than two glasses) and licorice (taken habitually), to reduce the impact of chromogenic substances[16,25];
- Smokers;
- Patients with orthodontic treatment;
- Use of CHX mouthwash within 30 days prior to enrolment[18];
- Use of systemic antibiotics within 3 months prior to enrolment[15,24,26];
- Allergy to substances used in this study.

The exclusion criteria were designed to standardize the sample and avoid extrinsic pigmentations due to the intake of chromogenic substances during mouth rinses use, as suggested by international literature.[21]

2.3 | Interventions

All the patients subscribed an informed consent and underwent to the same treatment. The same operator performed all treatments and all outcome measurements (GI, Plaque Control Record and Gingival Bleeding Index).

At baseline (T0), clinical indices Plaque Control Record (PCR—O'Leary Index)[27] and Gingival Bleeding Index (GBI—Ainamo Bay),[28] intraoral digital and spectrophotometric photographs were recorded for each patient. All patients underwent professional oral hygiene and selective polishing, with rotating bristle brush and prophylactic paste. In the same session, when professional oral hygiene was completed (T1), digital and spectrophotometric photographs were collected again; each patient received motivation and instruction of oral hygiene; they were randomly assigned to Group ADS, CHX or CPC to receive a mouthwash. The instructions to use the mouthwash have been explained and prescribed in the same way to each patient, as follows: oral rinse with 10 mL for 1 minute, twice a day, 30 minutes after tooth brushing, for 14 days, according to manufacturer instructions.

At day 14 (T2), clinical indices were taken, intraoral digital and spectrophotometric photographs were recorded; the feedback questionnaire about patients' perception of the used mouthwash was submitted to each patient. Selective polishing and motivation to oral hygiene were repeated.

2.4 | Outcomes

Primary outcomes were plaque and gingival bleeding; they were assessed according respectively to Plaque Control Record and Gingival Bleeding Index:

- Plaque Control Record (PCR—O'Leary Index).[27] A dental hygienist, using the PCP UNC 15 probe sliding along the cervical surface of all teeth, detected the presence of plaque in six points of tooth surface (disto-buccal, mesio-buccal, buccal and lingual, mesio-lingual, disto-lingual). The number of surfaces with plaque divided by the number of available tooth surfaces and multiplied by 100 expresses the percentage of plaque presence.
- Gingival Bleeding Index (GBI; Ainamo and Bay[28]). It detects the presence of gingival bleeding on gentle probing six points of dental surface (disto-buccal, mesio-buccal, buccal and lingual, mesio-lingual, disto-lingual). A dental hygienist, using the PCP UNC 15 probe sliding along the cervical surface of all teeth, assigned a positive score when bleeding occurs within 10-15 seconds. The number of positive areas was divided by the number of those examined, and the result was multiplied by 100 to express the index as a percentage.[29] The absence/reduction of Gingival Bleeding Index has been interpreted as an improvement of the inflammatory condition.

Secondary outcomes were adverse events: patients' perception and stains. A feedback questionnaire evaluated patients' perception: it contains six questions about bleeding reduction perception, alterations in food taste, alterations in perception of salt, burning sensation, dryness sensation and mouthwash taste.[13] The questionnaire only allowed yes/no answers. A spectrophotometer assessed mouthwashes staining effect comparing spectrophotometric quantitative colour difference (ΔE) on vestibular surfaces of central incisors before and after mouthwash treatment (T1-T2) (SpectroShade, MICRO, Serial N HDL1407, MHT, Arbizzano di Negrar, Verona, Italy).

Spectrophotometric measurements before and after treatment were performed on a black background on central incisors vestibular surfaces. The CIE-$L^*a^*b^*$ spectrophotometric coordinates,[30] calculated by the MHT software according to Guerra et al,[31] were used to evaluate the colour difference (ΔE) between T1 and T2. The ΔE that quantitatively assesses the colorimetric shade variation before and after mouthwash treatment (T1-T2) for each tooth was calculated using the following formula: $\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$, where, L_1, a_1, b_1 represent CIE-$L^*a^*b^*$ values before mouthwash use (T1), while L_2, a_2, b_2 are CIE-$L^*a^*b^*$ values after 14 days of mouthwash treatment (T2).

Referring to scientific literature, we defined the acceptability (AT) and perceptibility thresholds (PT) for colour differences respectively at 1.1 and 3.3 ($\Delta E > 3.3$ indicates a detectable colour difference beyond acceptability for human eye perception, $\Delta E < 1.1$ indicates no perceptible difference).[31]

2.5 | Sample size

The sample size was defined in 22 patients per Group, to have a two-sided 5% significance level and a power of 80%, given an anticipated dropout rate of 10%. Sample size was calculated in agreement with a pilot study in which we enrolled 40 patients that underwent the same treatment of this study. Based on the bleeding index, the optimal sample size for evaluating the efficacy of mouthwash was 54 patients.

2.6 | Randomization

A computer-generated list of random numbers was used to allocate the participants in the three Groups. The randomization sequence was created using IBM SPSS Statistic Software 20.0 Windows, International Business Machines Corp. New Orchard Road Armonk, New York 10504 US based through a casual sample stratified by patients' sex.

In this study was used allocation concealment of the random sequence to the operator who performed patient's enrolment, informed consent signature, treatment with professional oral hygiene and outcomes assessment.

The IBM SPSS Statistic Software 20.0 for Windows generated the randomization list; a specialist in oral hygiene (blinded and calibrated at the baseline) performed patient enrolment, professional oral hygiene procedures and outcome assessment; another dental specialist performed assignment to each Group for mouthwash

treatment; and each patient received the mouthwash in an anonymous bottle according to the randomization list.

2.7 | Blinding

Treatments identity was blinded to the operator that performed patient enrolment and outcomes assessment, to the data analysts and to participants. Only the operator who performed Group assignment was aware of the allocated Group.

2.8 | Statistical analysis

Preliminarily, a descriptive statistical analysis of the collected data at T0 was performed.

The Kolmogorov-Smirnov test examined data distribution; it confirmed that the data are consistent with a normal distribution. Categorical variables were summarized using frequencies in absolute and percentage values; while, for continuous variables, were calculated the central tendency measures (mean, median, mode), variability indices (standard deviation) and, where appropriate, their confidence intervals at 95%. For continuous variables, we used the parametric Student's *t* test to compare two sample means. The *t* test was executed separately for each mouthwash, to analyse the data pre- and post-treatment. Variables expressed as variations between observation times (T0, T1, T2) were tested using 2-tailed Student's *t* test for paired data. The ANOVA test checked for a statistically significant difference between means variations among Groups. Tukey's post hoc was applied for multiple comparison. The chi-square test assessed the association between categorical and nominal variables (spectrophotometric analyses and adverse events). Subjective patient's perception variables were considered dichotomous variables so, with reference to the question, the value 1 indicated affirmative answer and the value 0 a negative answer.

Results were considered statistically significant when they occurred with a probability <0.05.

3 | RESULTS

In this randomized clinical trial, 66 patients were recruited (T0 and T1). In ADS Group (n = 22), there were 9 males and 13 females, aged from 18 to 40 years with an average of 29.6. In CHX Group (n = 22), there were 8 males and 14 females, aged from 20 to 40 years with an average of 28.3. In CPC Group (n = 22), there were 9 males and 13 females, aged from 18 to 40 years with an average of 30.1. Two patients dropped out (1 of Group ADS and 1 of Group CHX). Sixty-four patients completed the study (T2).

Table 1 shows a descriptive analysis of demographic characteristics for each Group at baseline (T0).

Table 2 reveals, for each mouthwash, plaque build-up in the fourteenth (14th) day of mouthwash use (T1-T2). At time T1 (after oral hygiene session), all plaque values are considered 0. The paired sample *t* test analysis of PCR mean variation shows an average of 30.67 (SD = 15.22; 95% CI 23.55-37.80; $P = 0.000$), 19.93 (SD = 11.03; 95% CI 14.90-24.95; $P = 0.000$) and 16.24 (SD = 15.35; 95% CI 9.60-22.88; $P = 0.000$) respectively in Groups ADS, CHX and CPC.

TABLE 1 Descriptive analysis of demographic characteristics for each Group at baseline (T0)

Groups	Age	Male	Female
ADS	29.70 ± 8.30	9 (40.9%)	13 (59.1%)
CHX	27.90 ± 6.89	8 (36.4%)	14 (63.6%)
CPC	29.48 ± 6.46	9 (40.9%)	13 (59.1%)

Furthermore, (Table 2) the ANOVA analysis revealed that differences found in PCR mean variations among the three Groups were statistically significant (PCR $P = 0.004$). Particularly, according to the Tukey's post hoc multiple comparisons, mouthwash of Group CHX showed more effectiveness of Group ADS with $P = 0.045$. Mouthwash in CPC Group was significantly more effective than the mouthwash in ADS Group in reducing plaque build-up (0.004). No statistically significant differences were found in this analysis between mouthwashes of Groups CHX and CPC in plaque build-up reduction ($P = 0.661$).

Table 3 shows that all the mouthwashes had significant efficacy in reducing GBI in treated patients. The paired sample *t* test analysis of GBI mean variation (T0-T2) shows an average of −9.82 (SD = 9.27; 95% CI −5.48 to 14.16; $P = 0.000$), −19.31 (SD = 11.33; 95% CI −14.15 to −24.47; $P = 0.000$) and −21.13 (SD = 12.56; 95% CI −15.70 to −26.56; $P = 0.000$) respectively in Groups ADS, CHX and CPC.

The ANOVA analysis and the Tukey's post hoc multiple comparisons (Table 3) showed the statistically significance in this GBI mean variations among the three Groups ($P = 0.004$). Mouthwash in Group CHX and Group CPC was significantly more effective than mouthwash in Group ADS in reducing bleeding (respectively $P = 0.024$ and $P = 0.005$). No differences were found in between mouthwashes of CHX and CPC Groups ($P = 0.853$).

Table 4 shows subjective patient's perception variables, such as reduction of bleeding perception, burning and dryness sensation, altered taste, mouthwash taste and alterations in salt perception. For all variables, except for dryness sensation, there is an association, in the three Groups, between mouthwash and patient's perception. Groups ADS and CPC have never expressed alterations in salt perception while in Group CHX almost a quarter of subjects experienced it.

About the spectrophotometric staining evaluation, ΔE was <1.1 in 47 teeth and in 24 teeth ΔE was >3.3. Although ADS and CPC Groups showed a bigger colour difference between before and after mouthwash treatment (23.8% and 25% >3.3) compared to CHX Group (7.14% >3.3), the chi-square test did not appear to be significant ($P = 0.11$); therefore, data distribution could be casual.

4 | DISCUSSION

The results obtained show that 0.12% CHX + CPC mouthwash has the same efficacy of 0.2% CHX, in plaque and gingival bleeding

TABLE 2 Plaque Control Record timeline and mean variations between T1 and T2 by mouthwash and ANOVA and Tukey's post hoc multiple comparisons test

Plaque Control Record											
					Mean variation between times			Mean variation comparison between Groups			
										Multiple comparisons	
	N	T0	T1	T2	Mean (T1-T2)	*P*-value	95% CI	*F*	*P*-value		*P*-value
ADS Group	21	62.67 ± 16.87	0	30.67 ± 15.22	30.67 ± 15.22	0.000	23.55-37.80	5.984	0.004	ADS Group vs CHX Group	0.045
CHX Group	21	55.17 ± 14.31	0	19.93 ± 11.03	19.93 ± 11.03	0.000	14.91-24.95			ADS Group vs CPC Group	0.004
CPC Group	22	60.04 ± 13.15	0	16.24 ± 15.35	16.24 ± 15.35[a]	0.000	9.60-22.88			CHX Group vs CPC Group	NS

At time T1 (after oral hygiene session), all plaque values are considered 0. The lower values in mean variation between times indicate a minor plaque build-up.
NS, not significant.
[a]Best performance.

TABLE 3 Gingival Bleeding Index timeline and mean variations between T0 and T2 by mouthwash and ANOVA and Tukey's post hoc multiple comparisons test

Gingival Bleeding Index											
					Mean variation between times			Mean variation comparison between Groups			
										Multiple comparisons	
	N	T0	T1	T2	Mean (T0-T2)	*P*-value	95% CI	*F*	*P*-value		*P*-value
ADS Group	21	21.50 ± 12.17	/	11.67 ± 8.07	−9.82 ± 9.27	0.000	−5.48 to 14.16	6.097	0.004	ADS Group vs CHX Group	0.024
CHX Group	21	30.93 ± 20.75	/	11.62 ± 10.95	−19.31 ± 11.33	0.000	−14.15 to 24.47			ADS Group vs CPC Group	0.005
CPC Group	22	26.78 ± 13.52	/	5.65 ± 5.62	−21.13 ± 12.56[a]	0.000	−15.70 to 26.56			CHX Group vs CPC Group	NS

The lower values in mean variation between times indicate a greater reduction in bleeding.
NS, not significant.
[a]Best performance.

TABLE 4 Subjective patient's perception variables distribution. It indicates the percentage of positive responses to the questionnaire

	Mouthwashes			Pearson's Chi-Square
Questions	Group 1	Group 2	Group 3	*P*-value
Reduction of bleeding perception (%)	70.0	66.7	95.5[a]	0.046
Burning sensation (%)	15.0	52.4	13.6[a]	0.006
Dryness sensation (%)	30.0	42.9	18.2[a]	NS
Altered taste (%)	5.0[a]	57.1	9.1	0.000
Mouthwash taste (%)	35.0	23.8	100.0[a]	0.000
Salt perception (%)	0[a]	23.8	0[a]	0.006

NS, not significant.
[a]Best performance.

reduction. Both mouthwashes mentioned above, in terms of efficacy in plaque and gingival bleeding reduction, were >0.2% CHX + ADS mouthwash. The lower efficacy of CHX seems to be proper related to the addition of the ADS in mouthwash formulation, especially considering that it has the same CHX percentage of the 0.2% CHX mouthwash and the higher percentage of 0.12% CHX + CPC mouthwash.

International literature on CHX mouthwashes reports uneven results.[32,33] This may depend on the sample size or on the oral hygiene indications given to the patients. Obviously, studies that left patient to his daily oral hygiene habits will introduce inevitable bias that can thus affect the plaque-dependent gingivitis.

The CPC's ability to maximize CHX efficacy is demonstrated by Sreenivasan; both CPC rinses used showed >90% reductions in the viability of dental plaque complex than CHX and fluoride control rinses.[17] Quirynen et al[15] demonstrated the potential of the CHX 0.12% + CPC 0.05% in reducing of plaque and bleeding resulting as effective as CHX 0.20% + alcohol. In contrast, in the study of Najafi it seems to be a significant difference between CHX 0.12% and CHX 0.20% only in reducing gingival bleeding, proving to be CHX 0.12% less effective.[34]

Literature results on regards ADS role in reduction of CHX efficacy are contradictory.

Cortellini et al[13] showed that CHX + ADS was as effective as CHX without ADS in reducing gingival signs of inflammation in the post-surgical early healing phase. Graziani et al[35] in their study concluded that chlorhexidine-based mouthwash with ADS appeared less effective in terms of plaque reduction, compared to conventional CHX mouthwash, instead it showed a higher control of gingival inflammation. Also Solis et al,[36] when comparing CHX 0.20% + ADS vs CHX 0.20%, observed similar effectiveness in reducing plaque and bleeding. The in vitro study of Addy et al[16] revealed the same efficacy between CHX + ADS and CHX alone. According to Li et al,[26] the ability of 0.2% CHX with ADS to prevent plaque accumulation and gingivitis is highly questionable. Guggenheim[37] argues that, until now, it has not been possible to formulate CHX products with effective ADS additives without reducing antimicrobial activity. Even Arweiler et al[38] in their study reach the same conclusions; however, it must be emphasized the different formulations, in fact mouthwash with only CHX contained a high percentage of alcohol, unlike the mouthwash with CHX + ADS resulting free.

Regarding side effects, comparing the feedback questionnaire on the subjective patient's perception variables, 0.12% CHX + CPC mouthwash had fewer side effects than 0.2% CHX alone. In fact, from the general point of view, 100% of patients who used 0.12% CHX + CPC has enjoyed the pleasantness of the product versus 24% of patients that used 0.2% CHX mouth rinses.

Comparing the subjective perceptions of patients treated with 0.2% CHX and 0.2% CHX + ADS, the latter was more tolerable, especially about taste alteration that occurred in only 5% of cases compared with 57% of Group CHX. Data collected from patient's perception questionnaire, show better perception results (less side effects) of CHX 0.12%, maybe due to CHX lower concentration: bleeding reduction(the biggest part of CPC Group, three-quarters of the ADS Group, slightly more than 50% in CHX Group), burning sensation (a half of CHX Group, a quarter of CPC and ADS Groups) and dryness sensation (less than a quarter of CPC Group, more than a quarter of ADS Group and almost a half of CHX Group). The worse performance on salt perception was on CHX Group (a quarter of patients); it was not perceived in ADS and CPC Groups.

From the aesthetic point of view, in the present study, the spectrophotometer did not detect any significant differences between the three mouthwashes in their pigmentation effect. Therefore, we can state that the anti-stain molecule, added to the formulation of one of the mouthwashes, did not reduce pigmentation in comparison with mouthwashes without it. However, further analysis is necessary in cosmetic staining evaluation, since the spectrophotometer may have difficulty in colorimetric evaluation of interproximal surfaces.

In the study of Quirynen et al,[15] CHX 0.12% + CPC 0.05% seems to reduce the unpleasant side effects associated with CHX and alcohol as burning sensation or altered taste. Cortellini et al[13] specified that CHX + ADS caused less pigmentation than CHX without ADS; moreover, CHX + ADS mouthwash compared to only CHX rinse revealed best compliance, less burning and it was tolerated better. Graziani et al[35] associated the less staining to higher plaque levels obtained with mouthwash with ADS. Also Solis et al,[36] when comparing CHX 0.20% + ADS vs CHX 0.20%, observed less staining but no difference was detected in side effects. The

study of Addy et al[16] showed no significant difference in staining between CHX + ADS and CHX alone. Li et al[26] found that CHX with ADS did not completely eliminate the side-effect of staining.

Overall, despite the lowest CHX rate, 0.12% CHX + CPC mouthwash performed better than ADS and CHX Groups in plaque and bleeding reduction, showing also fewer side effects, in our opinion, due to the small percentage of CHX than the other two mouthwashes.

A limitation in our study is the not verified compliance to rising and to oral hygiene. Also, the absence of a negative control Group can be considered a limitation; however, the international literature has already shown that chlorhexidine is effective, so we are not testing chlorhexidine but the effect of two other different molecules on it that are ADS and CPC. We use 0.12% CHX in CPC Group, although this factor may be perceived as a limitation, 0.12% has been chosen instead of 0.20% because the latter is marketed only in the form of spray.

5 | CONCLUSIONS

About mouthwash effectiveness, each Group showed improvements of clinical indices after treatment, confirming what the international literature has already stated.

In this study, if all three mouthwashes had good clinical efficacy, compared to only CHX at the highest dosage, the addition of ADS showed a limited ability to reduce bacterial plaque and gingival bleeding, while addition of CPC showed a clinical efficacy not significantly different and less adverse events.

This is an important finding for patient compliance to therapeutic guidelines. We plan to extend the sample for more meaningful results especially for spectrophotometric pigmentation assessment.

6 | CLINICAL RELEVANCE

6.1 | Scientific rationale for study

This study intends to find out what would be the effect of the addition of ADS or CPC in CHX-based mouthwashes, in treatment of oral bleeding and gingivitis.

6.2 | Principal findings

This study demonstrated that adding CPC allows decreasing CHX concentrations and still achieves the same effects reducing plaque build-up and bleeding. Addiction of ADS instead demonstrated to be less efficacious than the solo CHX.

6.3 | Practical implications

The clinical rational is to guide the clinical practice to choose a mouthwash, according to benefits and side effects of CHX with or without the addiction of ADS or CPC.

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