Original article

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Antiseptic Capacity of 0.05% Chlorhexidine Digluconate and 0.05% Cetylpyridinium Chloride. A Prospective and Microbiological Clinical Study

ABSTRACT

Introduction: The aim of this study was to evaluate the clinical and microbiological efficacy of an oral rinse (colutory) containing 0.05% chlorhexidine digluconate (CHX) and 0.05% cetylpyridinium chloride (CPC) when compared to another rinse without antiseptic properties, used as adjuvants to oral hygiene methods.

Material and methods: Using spectrophotometry, a microbiological study was conducted to evaluate the capacity of each rinse to inhibit the formation and adhesion of Streptococcus oralis bacterial biofilm, followed by a randomized and double-blind clinical trial conducted on a sample of 48 patients randomly assigned to either rinse.

• Oral Rinse A: 0.05% CHX, 0.05% CPC and 0.14% zinc lactate

• Oral Rinse B: 0.10% permethol and 0.50% provitamin B5.

The plaque index (PI), modified gingival index (MGI), and sulcus bleeding index (SBI) were evaluated at one and three months.

Results: The 0.05% CHX and 0.05% CPC-based mouth rinse showed a high capacity to inhibit formation (P=0.013) and adhesion (P=0.001) of bacterial biofilm. Statistically significant differences in inter-group PI were observed at three months of observation (P<0.001). Differences in the MGI were also observed at one month (P=0.034) and three months of observation (P<0.001); and in the SBI at one month (P=0.004) and at three months of observation (P=0.002).

Conclusions: The 0.05% CHX and 0.05% CPC-based colutory

KEYWORDS

Chlorhexidine gluconate; Oral mouth rinse; Cetylpyridinium chloride; Gingivitis; Dental plaque.

INTRODUCTION

In recent years, molecular characterization of oral microbiota has succeeded in detecting 700 bacterial species or biotypes capable of colonizing the tissues of the oral cavity. However, a healthy individual is home to approximately 150 to 200 different bacterial species, of which between 10 to 30 have the capacity to cause periodontal disease (PD)[1]. Subgingival bacterial counts indicate that a healthy individual hosts 103 colony-forming units (CFUs), while in individuals with established PD this may amount to 108 CFUs[2]. These types of infections are responsible for causing damage to supporting tissues[3-]). Periodontal pathologies are characterized by their high prevalence. Epidemiological studies indicate that between 5 and 20% of the population suffers from advanced forms of periodontitis[6,7]. Periodontal disease etiopathogenesis is strongly associated with the formation of dental plaque. Dental plaque is a complex form of polymicrobial biofilm, a process which begins with the establishment, fixation, and growth of primary colonizing tooth bacteria, such as Streptococcus oralis.

Longitudinal clinical trials have shown that proper control of bacterial plaque prevents periodontitis, and that the lack of proper oral hygiene is accompanied by an increase in bacterial plaque and the onset of such pathology[8-10]. Numerous authors emphasize the importance of oral hygiene practices in preventing the formation of bacterial biofilm[11], as well as the use of oral antiseptics as adjuvants to such techniques[12].

Chlorhexidine digluconate (CHX) is the most widely used antiseptic agent today. It has been shown to be effective in preventing bacterial plaque formation and gingival inflammation[13,14]. The lack of controlled clinical studies evaluating the antiseptic capacity of 0.05% CHX and 0.05% cetylpyridinium chloride (CPC) to reduce or prevent the onset of disease in patients without PD clearly indicated the need for a prospective and microbiological clinical study to determine its effectiveness.

MATERIAL AND METHODS

MICROBIOLOGICAL STUDY
Study Design

The ability of two oral rinses to inhibit adhesion and formation of bacterial biofilm in vitro was analyzed:

- A: 0.05% CHX, 0.05% CPC and 0.14% zinc lactate (CN 323923.3. Halita®, Dentaid, Cerdanyola, Barcelona, Spain), and

- B: 0.10% Permethol and 0.50% Provitamin B5 (CN 350447.8. Parogencyl®, Procter & Gamble, Cincinnati, OH, USA).

Streptococcus oralis is a primary colonizer of tooth enamel and the cellular base on which future dental plaque forms. The adhesion and formation of bacterial biofilm was determined and quantified using spectrophotometric methodology. Polystyrene microtiter plates with 96 wells were used (Masterlab S.L., Madrid, Spain), in which a 175µl bacterial inoculum of S. oralis (obtained from an earlier study[15] suspended in trypticase soy broth was placed (specifically, TSB 150µl broth + 25µl of bacterial suspension adjusted to 0.5 McFarland density), then 25µl of the designated rinse was added to each of the wells.

Ability to inhibit bacterial biofilm formation

In this trial, each rinse under study (25µl) was added in serial dilutions ranging from 1/16 to 1 (undiluted) as a supernatant (25µl of rinse) to the bacterial inoculum (25µl of bacterial suspension in sterile saline solution) and 150µl of culture broth in the microtiter plate wells.

Ability to inhibit the adhesion of bacterial biofilm

For this test, each rinse was placed in the wells (25µl in dilutions ranging from 1/16 to 1) in advance while maintaining one minute contact with the surface of the plate by means of a microtiter plate mixer, decanted, and then the strain of S. oralis (150µl growth medium + 25µl bacterial inoculum) was inserted.

For the two tests in question, following 24 hours of incubation at 37ºC, the content of the wells was remo-

ved by decanting, and the plates were washed with sterile distilled water. The plates were dried at room temperature for 30 minutes and then each well was filled with 200μl of crystal violet dye (25%). After 5 minutes, the excess dye was removed by decanting, and the plate was washed again. After 30 minutes, 200ml of hydrochloric acid (25%) was added to the wells, and after 1 minute, the optical density (OD) of the bacterial biofilm that had adhered to the walls of the well was measured with a spectrophotometer (Labsystems Multiskan, Helsinki, Finland) at 450 nm. The tests were repeated four times, and the average readings of the ODs obtained, +/- their standard deviations, were used for comparison. Likewise, in each plate and test performed, the biofilm formed by S. oralis was measured via spectrophotometry in a series of eight inoculum wells without a rinse (basal biofilm), and in another series of wells the optical density obtained with broth without inoculum was measured (negative control).

Taking the spectrophotometric measures of absorbance obtained at 450nm, four values or cut-off points were established to evaluate the ability of the oral rinses to inhibit bacterial biofilm:

- No biofilm formation: OD: 0-0.059 to 450 nm.

- Weak biofilm formation: OD: 0.060-0.150 to 450 nm.

- Moderate biofilm formation: OD: 0.151-0.250 to 450 nm.

- High biofilm formation: OD>0.250 to 450 nm.

CLINICAL STUDY: Study Design

A clinical, randomized, double-blind trial was conducted on a sample of 48 patients. A 3-month observation period was set up during which reviews were scheduled following one and three months after patients joined the study. The study was conducted in accordance with the ethical principles set out in the Helsinki Declaration, following the guidelines of Good Clinical Practice. The study was carried out at the Alfonso X el Sabio University Dental Clinic in Madrid between June and July 2017. An informed consent form was drafted which was evaluated by the Ethics Committee of the "Gómez Ulla" Hospital Central de la Defensa in Madrid. Each patient was randomly assigned (Epidata version 3.1, PAHO/WHO, A Coruña, Spain) to one of the oral rinses:

- A: 0.05% CHX, 0.05% CPC and 0.14% zinc lactate (CN 323923.3. Halita®, Dentaid, Cerdanyola, Barcelona, Spain), and

- B: 0.10% Permethol and 0.50% Provitamin B5 (CN 350447.8. Parogencyl®, Procter & Gamble, Cincinnati, OH, USA).

Selection Criteria

The study inclusion criteria consisted of patients over the age of 18 who had more than 20 testable teeth. The study exclusion criteria consisted of: loss of bone insertion greater than 1/3 of root length, presence of periodontal sacks equal to or greater than 4 mm, individuals undergoing orthodontic treatment or periodontal treatment in the last 3 months, individuals being treated with antibiotics or corticosteroids in the last month, hypersensitivity to any of the study components, smokers with a consumption level greater than 10 cigarettes/day, pregnant or nursing women, patients with Sjogren's syndrome and patients with systemic diseases.

Clinical Procedure

The following clinical indices were analyzed: bacterial plaque index (PI)[16] to evaluate the antiplaque property of the rinses, and the modified gingival index (MGI)[17] and sulcus bleeding index (SBI)[18] to analyze the degree of gingival inflammation. The extrinsic stain index (SI)[19] was included to record possible dyschromia derived from the rinses.

After evaluating PI, MGI, SBI and SI during the initial visit, mechanical removal bacterial plaque was performed using ultrasound (SONICflex quick 2008 L, KaVo®, Benelux, France) and prophylaxis paste (Mira Clean®, Hager & Werken, Duisburg, Germany) employing low-speed rotary instruments (GENTLEPOWER LUX 20 LP, KaVo®, Benelux, France). Each patient was given a kit with oral hygiene products consisting of toothpaste

(CN 150331.2. Sodium fluoride (1450 ppm ion fluoride), vitamin E and xylitol (Dentaid, Cerdanyola, Spain); an intermediate hardness toothbrush (CN 154054.6. (Dentaid, Cerdanyola, Spain); dental floss (CN 332890.6 Dentaid, Cerdanyola, Spain); and the randomly assigned oral rinse (A or B). Patients were also instructed in oral hygiene techniques in order to homogenize both oral hygiene techniques and the products used. After using the oral rinse, volunteers were instructed to refrain from any additional rinsing and eating any food for the following 30 minutes. They were also instructed to avoid using any other method or means of oral hygiene.

The PI, MGI, SBI and SI were measured during the visits at one and three months.

The information obtained from each patient was logged and archived in a dossier in conformity with Organic Law 15/1999 on the Protection of Personal Data.

STATISTICAL ANALYSIS

As measures of central tendency, the arithmetic mean (M) and standard deviation (SD), or the median (θ) and interquartile range (IQR) were used.

Mann Whitney's U-test was used. In all cases, a p<0.05 value was taken as the cutoff value for statistical significance. SPSS® v15 software (Microsoft Inc., Redmond, WA, USA) was used as the statistical application.

RESULTS

MICROBIOLOGICAL STUDY

Ability to inhibit bacterial biofilm formation

Oral rinse A showed a greater ability to inhibit the formation of bacterial biofilm (P=0.013), than did oral rinse B (P=0.280) (Figure 1).

Ability to inhibit the adhesion of bacterial biofilm

Both oral rinse A (P=0.001) and B (P=0.001) were shown to be effective in inhibiting the adhesion of bacterial biofilm (Figure 2).

CLINICAL STUDY

The average age of the study subjects was 36.4 with a standard deviation of 10.5; the minimum age was 20 and the maximum 57. Group assignment was conducted randomly and properly weighted for gender (P=0.303); however, statistically significant differences by age (P=0.031) were observed, which were considered clinically irrelevant. In the case of group B there was one volunteer who left the study after one month of observation.
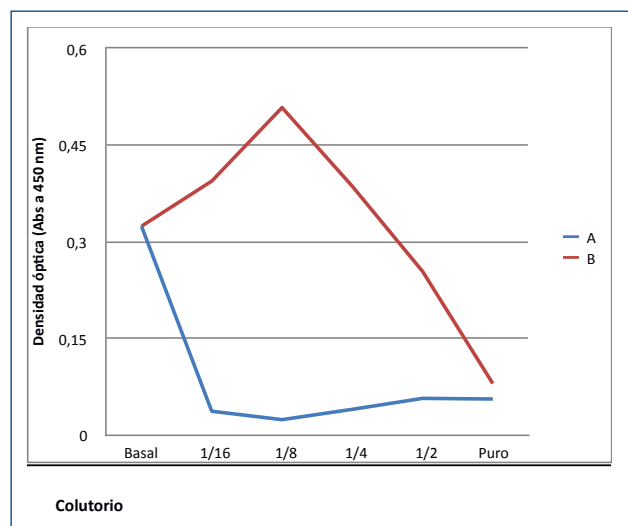

Figure 1. Ability to inhibit the formation of bacterial biofilm.
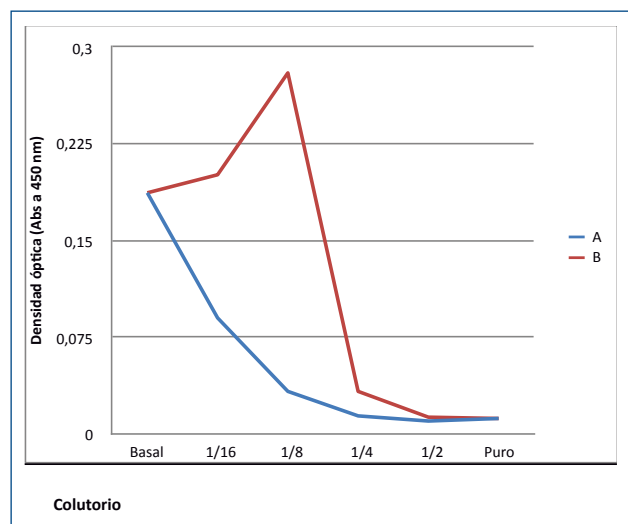

Figure 2. Ability to inhibit the adhesion of bacterial biofilm

No statistically significant differences were found in the variables collected in the medical (P=0.261) or dental history of the two groups (P=0.360).

The median PI value of Group A was reduced from an initial value of 2.5(1) to 2(1) at 30 days and to 1(0) at 90 days, while the median PI value of Group B was reduced from an initial value of 2(1.75) to 2(1) at 30 days and to 2(0) at 90 days. No statistically significant differences in initial PI (P=0.095) were observed, nor in the PI at one month of observation (P=0.205). However, differences between the two groups (P<0.001) were observed at three months (Table 1). Also, there were differences in the percentage of PI reduction between the two groups at one month (P=0.026) and at three months (P<0.001) (Table 2).

The median value of MGI for Group A was unchanged during the observation period, 1(1) while the median value of MGI for Group B was reduced at one month to 1(1) from the initial value of 2(1) and increased to 2(1) at three months. No statistically significant differences were observed in the initial MGI (P=0.269). However, differences were observed at one (P=0.034) and three months between the two groups (P<0.001) (Table 3). There was no difference in the percentage of MGI re-duction at one month (P=0.180); however, differences were observed between the two groups at three months (P=0.021) (Table 4).

The median SBI value for Group A was reduced from an initial value of 1(1) to 0(0) at 30 and 90 days, while the median value for Group B was reduced from an initial value of 1(2)to 0(1)at 30 and 90 days. No statistically significant differences were observed in the initial SBI (P=0.556); however, differences between the two groups were observed at one (P=0.004) and three months (P=0.002) (Table 5).

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Differences in the percentage of SI reduction between the two groups at one (P=0.026) and three months (P<0.001) were also observed. No differences were observed in the percentage reduction of SI between the two groups at one month (P=0.053). However, there differences did arise between the two at 3 months (P=0.026) (Table 6). The SI did not show dyschromia attributable to exposure to the oral rinses during the observation period.

TABLE 1. BACTERIAL PLAQUE INDEX (PI).

ORAL RINSE			INITIAL VISIT	FIRST MONTH	THIRD MONTH
A	N	Valid	24	24	24
		Lost	0	0	0
	Percentile	25	2.00	1.00	1.00
		50	2.50	2.00	1.00
		75	3.00	2.00	1.00
B	N	Valid	24	23	23
		Lost	0	1	1
	Percentile	25	1.25	1.00	2.00
		50	2.00	2.00	2.00
		75	3.00	2.00	2.00
p			0.095	0.205	<0.001

Table 2. Percentage reduction in the bacterial plaque index (PI).

	ORAL RINSE A		ORAL RINSE B		
	IP Variation	Frequency (valid percentage)	IP Variation	Frequency (valid percentage)	Sig. bilateral
FIRST MONTH	Increase 1 point	-	Increase 1 point	2 (8.7%)	
	It stays	5 (20.8%)	It stays	13 (56.5%)	
	Reduce 1 point	14 (58.3%)	Reduce 1 point	7 (30.4%)	
	Reduce 2 points	5 (20.8%)	Reduce 2 points	1 (4.3%)	0.026
	Total	24 (100%)	Total	23 (100%)	
	Lost System		Lost System	1	
	Total	24	Total	23 (100%)	
THIRD MONTH	Increase 1 point	-	Increase 1 point	6 (26.1%)	
	It stays	1 (4.2%)	It stays	11 (47.8%)	
	Reduce 1 point	13 (54.2%)	Reduce 1 point	5 (21.7%)	
	Reduce 2 points	9 (37.5%)	Reduce 2 points	1 (4.2%)	<0.001
	Reduce 3 points	1 (4.2%)	Reduce 3 points	-	
	Total	24 (100%)	Total 23 (100%)		
	Lost System		Lost System	1	
	Total		Total	24	

Table 3. Modified gingival index (MGI).

ORAL RINSE			INITIAL VISIT	FIRST MONTH	THIRD MONTH
A	N	Valid	24	24	24
		Lost	0	0	0
	Percentile	25	2.00	1.00	1.00
		50	2.50	2.00	1.00
		75	3.00	2.00	1.00
B	N	Valid	24	23	23
		Lost	0	1	1
	Percentile	25	1.00	1.00	1.00
		50	2.00	1.00	2.00
		75	2.00	2.00	2.00
P			0.269	0.034	<0.001

No adverse effects were observed in any of the groups during the observation period.

DISCUSSION

The objective of this study was to compare the clinical and microbiological efficacy of 0.05% CHX and 0.05% CPC as an adjuvant agent for oral hygiene methods with respect to a control rinse without antiseptic properties. The clinical efficacy and adverse effects of CHX-based oral antiseptics are associated with their concentration: starting at 0.2% their clinical efficacy no longer increases, but the occurrence of adverse effects does[20,21], while a concentration of less than 0.05% is insufficient to be effective. Long-term clinical studies highlight the clinical efficacy of CHX 0.12% in reducing

PI and Gingival Index (GI) between 36.1 to 60.9% and 29.1 to 37.2% respectively[22,23]. These results differ from those obtained in our study because they do not involve manual hygiene techniques, employ lower sensitivity clinical indices, and do not include CPC in the composition of the antiseptic agent.

CPC exerts synergistic action in combination with CHX, performing its action at the level of the bacterial cell membrane and thereby causing a disruption in cell metabolism, inhibition of cell growth, and cell death[24]. The incorporation of CPC into CHX antiseptics has served to reduce its effective concentration and the occurrence of adverse effects without diminishing clinical efficacy[20]. Santos et al. (2004) evaluated the clinical and microbiological activity of an antiseptic based on 0.05% CHX and 0.05% CPC, observing a

TABLE 4. PERCENTAGE REDUCTION IN THE MODIFIED GINGIVAL INDEX (MGI).

	ORAL RINSE A		ORAL RINSE B		
	IGM Variation	Frequency (valid percentage)	IGM Variation	Frequency (valid percentage)	Sig. bilateral
FIRST MONTH	Increase 1 point	-	Increase 1 point	-	0.180
	It stays	9 (37.5%)	It stays	15 (65.2%)	
	Reduce 1 point	13 (54.2%)	Reduce 1 point	6 (26.1%)	
	Reduce 2 points	2 (8.3%)	Reduce 2 points	2 (8.7%)	
	Total	24 (100%)	Total	23 (100%)	
	Lost System		Lost System	1	
	Total		Total	24	
THIRD MONTH	Increase 1 point	-	Increase 1 point	5 (21.7%)	0.021
	It stays	10 (41.7%)	It stays	13 (56.5%)	
	Reduce 1 point	10 (41.7%)	Reduce 1 point	5 (21.7%)	
	Reduce 2 points	4 (16.7%)	Reduce 2 points	-	
	Reduce 3 points	-	Reduce 3 points	-	
	Total	24 (100%)	Total	23 (100%)	
	Lost System		Lost System	1	
	Total		Total	24	

Table 5. Sulcus Bleeding Index (SBI).

ORAL RINSE			INITIAL VISIT	FIRST MONTH	THIRD MONTH
A	N	Valid	24	24	24
		Lost	0	0	0
	Percentiles	25	0.00	0.00	0.00
		50	1.00	0.00	0.00
		75	1.00	0.00	0.00
B	N	Valid	24	23	23
		Lost	0	1	1
	Percentiles	25	0.00	0.00	0.00
		50	1.00	0.00	0.00
		75	2.00	1.00	1.00
P			0.556	0.004	0.002

Table 6. Percentage reduction in Sulcus Bleeding Index (SBI).

	ORAL RINSE A		ORAL RINSE B		
	SBI Variation	Frequency (valid percentage)	SBI Variation	Frequency (valid percentage)	Sig bilateral
FIRST MONTH	1-point increase	-	1-point increase	1 (4.3%)	0.026
	No change	9 (37.5%)	No change	14 (60.9%)	
	1-point Decrease	13 (54.2%)	1-point Decrease	3 (13%)	
	2-point Decrease	2 (8.3%)	2-point Decrease	5 (21.7%)	
	Total	24 (100%)	Total	23 (100%)	
	Sample Loss		Sample Loss	1	
	Total		Total	24	
THIRD MONTH	1-point increase	1 (4.3%)	1-point increase	4 (17.5%)	<0.001
	No change	9 (37.5%)	No change	11 (47.8%)	
	1-point Decrease	12 (50%)	1-point Decrease	5 (21.7%)	
	2-point Decrease	2 (8.3%)	2-point Decrease	3 (13%)	
	Reduce 3 points	-	Reduce 3 points	-	
	Total	24 (100%)	Total	23 (100%)	
	Sample Loss		Sample Loss	1	
	Total		Total	24	

percentage reduction in PI and GI of 40.8% and 29.4% respectively[25]. These results contrast with those observed in patients assigned to study group A, who reduced their PI by 95.9% and MGI by 58.4% at the three-month mark. These differences are attributed to discrepancies in the observation period, the use of lower sensitivity clinical indices and differences in the sample selection criteria.

CONCLUSIONS

A solution of Chlorhexidine digluconate-based antiseptic at 0.05% and 0.05% cetylpyridinium chloride has demonstrated its clinical and microbiological efficacy by reducing bacterial plaque and gingivitis.

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