



Randomised Controlled Trial Comparing the Clinical Effectiveness of Mouthwashes Based on Essential Oils, Chlorhexidine, Hydrogen Peroxide and Prebiotic in Gingivitis Treatment

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Abstract

Aim: The present clinical study aimed to investigate the clinical efficacy of 5 types of mouthwash based on different active substances.

Materials and methods: The study included 180 patients divided into 6 groups of 30 patients, each group rinsing with one of the following types of mouthwash based on: essential oils, combination of essential oils and 0.12% chlorhexidine, hydrogen peroxide (0.8%), prebiotic, 0.2% chlorhexidine, and placebo. All participants underwent professional mechanical plaque removal after which they were instructed to rinse with 15 ml mouthwash 2 times a day for 21 days. During the study period, patients were monitored at days 0, 14, and 21, examining oral hygiene index, gingival index, bleeding index, and presence of side effects.

Results: Gingival index, bleeding index, and oral hygiene index were reduced statistically significantly in all treatment groups. Adjunctive use of mouthwashes demonstrated better clinical effectiveness compared to mechanical plaque control (and placebo mouthwash). The gingival index and the plaque index were reduced most significantly in the group using mouthwash with hydrogen peroxide. The bleeding index decrease was most significant in the group using 0.2% chlorhexidine.

Conclusions: All tested mouthwashes demonstrated significant clinical effectiveness in different degrees in gingivitis treatment. New formulas with prebiotic and combination of essential oils and chlorhexidine indicate promising effectiveness.

Keywords

chlorhexidine, essential oils, gingivitis, hydrogen peroxide, mouthwashes, prebiotics

INTRODUCTION

Gingivitis is a plaque-induced inflammatory response to the bacterial plaque accumulation around the gingival margin.^[1] Regarding the last classification of periodontal dis-

eases and conditions, a gingivitis case is a case with bleeding score more than 10%.^[2] As it is prerequisite for periodontitis development and is a completely reversible disease, its management is of primary importance.^[3,4] Gingivitis can be successfully treated by combination of activities that include

motivation and instruction for proper oral hygiene, professional mechanical plaque removal and subsequent application of anti-inflammatory oral care products.^[2]

In a recent systematic review and meta-analysis, Figueroa et al. reported that the adjunctive use of antiseptics leads to significant reduction of gingival inflammation.^[5] Different agents in a variety of delivery formats are available on the market, but the adjunctive use of rinses demonstrates better results in comparison to dentifrices.^[5] Clinically proven efficacy is possessed by essential oils^[6], chlorhexidine^[7], and cetylpyridinium chloride^[8]. Some of them possess antibacterial and antifungal effectiveness.^[9] Hydrogen peroxide in different concentrations is also reported as an anti-plaque agent.^[10] In recent years, prebiotics and probiotics have been used in the adjunctive treatment of gingivitis and periodontitis.^[11] Prebiotics are non-digestible food ingredients that favour the activity and the growth of beneficial microorganisms and thus could promote the prevention and treatment of oral diseases.^[12-14]

AIM

The aim of the present study was to evaluate the clinical effectiveness of different active agents – essential oils, combination of essential oils and 0.12% chlorhexidine, 0.2% chlorhexidine, prebiotic, and 0.8% hydrogen peroxide in the adjunctive gingivitis treatment.

MATERIALS AND METHODS

The study included 180 patients (53.25% female and 46.75% male) recruited by referral. The mean age of the participants is 27.16 ± 7.37 years. They were divided into 6 groups of 30 patients. Forty-three (23.9%) patients were smokers smoking 6.45 ± 7.28 cigarettes per day. All patients signed an informed consent prior to the examination. The study was conducted in the Department of Periodontology and Oral Mucosa Diseases, Faculty of Dental Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria from September 2020 to December 2020. Each group rinsed with one of the following types of mouthwash – mouthwash based on essential oils (menthol, thymol and eucalyptus, 24% alcohol) – group 1, mouthwash based on essential oils (menthol, thymol and eucalyptus, 14.5%) and 0.12% chlorhexidine – group 2, placebo (containing water, sweetener and flavoring) – group 3, mouthwash based on 0.2% chlorhexidine (without alcohol) – group 4, mouthwash based on prebiotic (inulin) – group 5, and mouthwash based on hydrogen peroxide (0.8%) – group 6. The mouthwashes were in a process of development and this was a Phase II clinical trial (detailed information about all the ingredients is available on request from the corresponding author). Inclusion criteria were: generalized gingival inflammation, plaque index (PI) >1.95 (modified Quigley & Hein Oral hygiene index of Turesky, 1970 – OHI^[15]), gin-

gival index (GI) >0.95 (Loe & Silness, 1963)^[16], no systemic diseases, no systemic medication, lack of severely damaged teeth, no large fillings, no orthodontic treatment. Exclusion criteria were: periodontitis, use of antimicrobial drugs in the last 6 months, pregnancy, and lactation. The patients were motivated and instructed to maintain proper and optimal personal oral hygiene using Bass brushing technique, interdental brushes and floss. All participants underwent a professional mechanical plaque removal. After instrumentation participants were instructed to rinse with 15 ml mouthwash for 30 seconds 2 times a day after mechanical plaque removal using toothbrush and toothpaste for 21 days. Researchers controlled the amount of mouthwash used by giving a new bottle of mouthwash with the required amount for 1 patient for 1 week at the beginning of each week and taking back the bottle from the previous week. During the study period, patients were monitored on days 14 and 21, examining plaque index (Turesky, 1970)^[15], gingival index (Löe & Silness, 1963)^[13], bleeding index (Animo & Bay, 1975)^[17], and the presence of side effects like staining, burning itching, oral lesions at the end of the study (day 21).

The statistical analysis was performed with IBM SPSS Statistics Ver.19.0.

The study was approved by the Ethical Committee of Medical University of Plovdiv (Protocol 7/01.10.2020).

RESULTS

The mean values for GI, BI and OHI at each appointment (initial – I, 14th day – II and 21st day – III) are presented in **Table 1**. They demonstrate clearly that two weeks after using the mouthwash (second visit), the lowest values of the gingival index (GI) were observed in group 6 (0.27), followed by group 5 (0.38), and group 2 (0.40). The achieved good results for the GI in these three groups were maintained at the third visit (day 21), when the lowest reported values of the gingival index were in groups 6 (GI – 0.18), 2 (GI – 0.24), and 5 (GI – 0.38).

Regarding the bleeding index (BI), a similar trend is observed, as on the second and third visit the lowest reported values were in group 5 (BI – 6.63% of the second and BI – 3, 71% on the third visit) and group 6 (BI -11.66% on the second and BI – 7.60% on the third visit). The next group with best values in the bleeding index was group 4 (BI – 12.53% on the second and BI – 11.33% on the third visit). In fourth place is group 2 – 19.30% at the second and 11.36% at the third visit.

At the third visit (after 21 days of water use) the lowest values of OHI were observed in group 1 (PI – 0.83), followed by group 4 (PI – 1.21).

The gingival index (GI) decreased statistically significantly for all groups within 21 days (third visit) (**Table 2**). The biggest reduction was observed in group 6 (0.8% hydrogen peroxide) – 1.31, with a more significant decrease in the first 14 days (second visit) of mouthwash usage

Table 1. Mean values of gingival index, bleeding index, and oral hygiene index for the first, second, and third visits

Index	Mouthwash	N	Mean	Std. Deviation	χ^2	Sig.
Gingival index – 1st visit	1	30	1.71	0.19	26.401	0.000
	2	30	1.11	0.17		
	3	30	1.24	0.24		
	4	30	1.62	0.23		
	5	30	1.35	0.34		
	6	30	1.50	0.26		
	Total	180	1.42	0.32		
Gingival index – 2nd visit	1	30	1.44	0.13	57.442	0.000
	2	30	0.40	0.23		
	3	30	0.52	0.32		
	4	30	0.83	0.55		
	5	30	0.38	0.24		
	6	30	0.27	0.25		
	Total	180	0.64	0.51		
Gingival index – 3rd visit	1	30	1.02	0.09	38.645	0.000
	2	30	0.24	0.12		
	3	30	0.42	0.25		
	4	30	0.79	0.56		
	5	30	0.38	0.28		
	6	30	0.18	0.14		
	Total	180	0.50	0.41		
Bleeding index – 1st visit	1	30	46.25	13.25	4.846	0.000
	2	30	46.27	13.93		
	3	30	41.62	12.54		
	4	30	61.43	22.83		
	5	30	47.88	16.92		
	6	30	52.27	20.3		
	Total	180	49.29	17.9		
Bleeding index – 2nd visit	1	30	21.68	5.92	9.083	0.000
	2	30	19.30	11.56		
	3	30	21.36	13.85		
	4	30	12.53	14.47		
	5	30	6.63	8.15		
	6	30	11.66	10.64		
	Total	180	15.53	12.37		
Bleeding index – 3rd visit	1	30	15.63	4.59	9.817	0.000
	2	30	11.36	6.06		
	3	30	17.58	10.51		
	4	30	11.33	14.31		
	5	30	3.71	6.78		
	6	30	7.60	7.47		
	Total	180	11.20	9.93		
Oral hygiene index – 1st visit	1	30	2.07	0.14	19.045	0.00
	2	30	2.84	0.44		
	3	30	2.13	0.20		
	4	30	2.38	0.46		
	5	30	2.55	0.30		
	6	30	2.71	0.60		
	Total	180	2.45	0.48		

Index	Mouthwash	N	Mean	Std. Deviation	χ^2	Sig.
Oral hygiene index – 2nd visit	1	30	1.11	0.22	22.898	0.00
	2	30	2.22	0.36		
	3	30	1.13	0.48		
	4	30	1.26	0.68		
	5	30	1.81	0.62		
	6	30	1.50	0.52		
	Total	180	1.51	0.64		
Oral hygiene index – 3rd visit	1	30	0.83	0.20	25.269	0.00
	2	30	2.03	0.22		
	3	30	1.09	0.47		
	4	30	1.21	0.66		
	5	30	1.36	0.41		
	6	30	1.37	0.50		
	Total	180	1.32	0.57		

Table 2. Dynamics of changes in the gingival index (GI) at the three visits

Mouthwash	GI between I and II; II and III; I and III visits	Mean value of the differences	Std. deviation	U	Sig.
1. Essential oils	GI – 1st visit – 2nd visit	0.27	0.23	6.265	0.00
	GI – 2nd visit – 3rd visit	0.42	0.15	15.917	0.00
	GI – 1st visit – 3rd visit	0.69	0.26	14.684	0.00
2. Essential oils + 0.12% CHX	GI – 1st visit – 2nd visit	0.70	0.15	25.194	0.00
	GI – 2nd visit – 3rd visit	0.16	0.13	6.7605	0.00
	GI – 1st visit – 3rd visit	0.87	0.11	41.79	0.00
3. Placebo	GI – 1st visit – 2nd visit	0.71	0.30	12.978	0.00
	GI – 2nd visit – 3rd visit	0.10	0.23	2.3977	0.02
	GI – 1st visit – 3rd visit	0.81	0.27	16.76	0.00
4. 0.2% CHX	GI – 1st visit – 2nd visit	0.79	0.46	9.324	0.00
	GI – 2nd visit – 3rd visit	0.05	0.20	1.252	0.22
	GI – 1st visit – 3rd visit	0.83	0.47	9.79	0.00
5. Prebiotic	GI – 1st visit – 2nd visit	0.97	0.44	12.124	0.00
	GI – 2nd visit – 3rd visit	0.01	0.20	0.1022	0.92
	GI – 1st visit – 3rd visit	0.97	0.46	11.506	0.00
6. 0.8% H ₂ O ₂	GI – 1st visit – 2nd visit	1.23	0.32	20.806	0.00
	GI – 2nd visit – 3rd visit	0.09	0.18	2.7247	0.01
	GI – 1st visit – 3rd visit	1.31	0.22	32.276	0.00

(1.23) compared to the initial visit. The smallest was the reduction of GI in group 1 (essential oils with high % of alcohol) – 0.69. For all other groups, the decrease was greater than 0.83, where in the groups using mouthwash based on 0.2% chlorhexidine and prebiotic, the main reduction was in the first two weeks.

Considering reduction of bleeding at the end of follow-up – the third visit compared to the first study, it was the highest in group 4 – 0.2% chlorhexidine (50.10%), followed by group 6 – hydrogen peroxide (44.67%) and group 5 – prebiotic (44.18%), and finally by group 2 – essential oils in combination with 0.12% chlorhexidine (34.91%)

and group 1 – only essential oils (30.62%) (Table 3). It is noteworthy that in groups in which patients used mouthwashes based on essential oils with and without the addition of chlorhexidine, the decrease of this index was more significant between the 14th and 21st day (by 7.94% and 6, 05%) compared to other mouthwashes where the decrease between the second and third visits in the bleeding index was less than 4%. The control group – 3 (placebo) the average values of the bleeding index were the highest compared to the other groups 21.36% at the second visit and 17.58% on the third visit.

The oral hygiene index (OHI) decreased significantly

at days 14 and 21 in all study groups compared to baseline ($p < 0.05$). The best reduction was demonstrated in group 6 – hydrogen peroxide (reduction is 1.34), followed by group 1 – essential oils (1.24). The decrease of the plaque index in

group 4 – 0.2% chlorhexidine and group 5 – prebiotic was also > 1 (1.17 and 1.19, respectively) (Table 4).

Neither of the groups reported staining of teeth after using the mouthwashes. Only a burning sensation was re-

Table 3. Dynamics of changes in the bleeding index (BI) at the three visits

Mouthwash	BI between I and II; II and III; I and III visits	Mean value of the differences	Std. deviation	U	Sig.
1. Essential oils	BI – 1st visit – 2nd visit	24.57	12.39	10.861	0.00
	BI – 2nd visit – 3rd visit	6.05	4.62	7.1758	0.00
	BI – 1st visit – 3rd visit	30.62	10.75	15.603	0.00
2. Essential oils + 0.12% CHX	BI – 1st visit – 2nd visit	26.97	9.21	16.048	0.00
	BI – 2nd visit – 3rd visit	7.94	6.58	6.6046	0.00
	BI – 1st visit – 3rd visit	34.91	10.15	18.84	0.00
3. Placebo	BI – 1st visit – 2nd visit	20.26	15.15	7.327	0.00
	BI – 2nd visit – 3rd visit	3.78	10.70	1.9345	0.06
	BI – 1st visit – 3rd visit	24.04	14.56	9.047	0.00
4. 0.2% CHX	BI – 1st visit – 2nd visit	48.88	16.72	16.021	0.00
	BI – 2nd visit – 3rd visit	1.2	3.22	2.0408	0.05
	BI – 1st visit – 3rd visit	50.10	17.95	15.283	0.00
5. Prebiotic	BI – 1st visit – 2nd visit	41.25	14.80	15.265	0.00
	BI – 2nd visit – 3rd visit	2.93	4.24	3.7815	0.00
	BI – 1st visit – 3rd visit	44.18	16.03	15.093	0.00
6. 0.8% H ₂ O ₂	BI – 1st visit – 2nd visit	40.61	19.84	11.211	0.00
	BI – 2nd visit – 3rd visit	4.06	6.30	3.5273	0.00
	BI – 1st visit – 3rd visit	44.67	18.72	13.072	0.00

Table 4. Dynamics of changes in the oral hygiene index (OHI) at the three visits

Mouthwash	OHI between I and II; II and III; I and III visits	Mean value of the differences	Std. deviation	U	Sig.
1. Essential oils	PI – 1st visit – 2nd visit	0.95	0.26	20.325	0.00
	PI – 2nd visit – 3rd visit	0.28	0.10	15.251	0.00
	PI – 1st visit – 3rd visit	1.24	0.24	27.883	0.00
2. Essential oils + 0.12% CHX	PI – 1st visit – 2nd visit	0.61	0.39	8.526	0.00
	PI – 2nd visit – 3rd visit	0.19	0.26	3.9527	0.00
	PI – 1st visit – 3rd visit	0.80	0.40	10.982	0.00
3. Placebo	PI – 1st visit – 2nd visit	1.00	0.47	11.719	0.00
	PI – 2nd visit – 3rd visit	0.05	0.32	0.7778	0.44
	PI – 1st visit – 3rd visit	1.05	0.50	11.397	0.00
4. 0.2% CHX	PI – 1st visit – 2nd visit	1.12	0.48	12.92	0.00
	PI – 2nd visit – 3rd visit	0.05	0.16	1.7441	0.09
	PI – 1st visit – 3rd visit	1.17	0.47	13.615	0.00
5. Prebiotic	PI – 1st visit – 2nd visit	0.74	0.77	5.293	0.00
	PI – 2nd visit – 3rd visit	0.45	0.52	4.7389	0.00
	PI – 1st visit – 3rd visit	1.19	0.55	11.803	0.00
6. 0.8 % H ₂ O ₂	PI – 1st visit – 2nd visit	1.21	0.51	13.123	0.00
	PI – 2nd visit – 3rd visit	0.13	0.37	1.9159	0.06
	PI – 1st visit – 3rd visit	1.34	0.55	13.372	0.00

ported in groups 1, 2, and 6, where in the first two groups, 30% of the participants reported this side effect, whereas in group 6, only 16% reported the adverse effect (Table 5).

DISCUSSION

The present study is conducted as an intermediate-length trial (2 weeks to 2 months), which allows the assessment of gingivitis.^[18] Five different active agents in the composition of mouthwashes were examined in the adjunctive treatment of gingivitis, and compared to placebo mouthrinse. The tested null hypothesis (H_0) states that the statistical significance between the effectiveness in the placebo group and groups with active substances is missing.

Gingival index reduces statistically significantly at the second and third appointments in all treatment groups compared to the initial visit. In the group using essential oils and high percentage alcohol, the decrease of GI was the smallest (Table 2). The reason could be the high percentage of alcohol that leads to erosions of the oral mucosa and redness of the gingiva, registered as one of the indicators in GI.^[19] Moreover, 11 of the patients treated in this group declared burning sensation during mouthwash usage.

According to recent studies, the bleeding index (BI) is considered to be the main index showing the stability of treatment and the absence of active disease.^[20] Various studies have shown that in order for a patient to switch to maintenance periodontal therapy, the bleeding index needs to be less than or equal to 15 to 30%. The present study clearly demonstrates that the control group, in which mechanical instrumentation is performed and patients use placebo mouthwash, the average values of the bleeding index were the highest compared to the other groups (21.36%

at the second visit and 17.58% on the third visit) (Table 1). This demonstrates that all mouthwashes contribute to a more stable periodontal condition when applied in addition to standard mechanical cleaning. Moreover in groups 5 – prebiotic and 6 – hydrogen peroxide, the bleeding index at the third visit was less than 10%, which means that these patients do not have gingivitis anymore but sites with gingival inflammation.^[2] In groups 2 – combination of essential oils and 0.12% chlorhexidine and 4 – 0.2% chlorhexidine, the percentage of BI was around 11%, which corresponds to localized gingivitis but is just above the threshold.

Plaque index reduction is the most significant in the group where patients used mouthwash with 0.8% hydrogen peroxide. The effectiveness of hydrogen peroxide in different concentrations is controversial.^[18] In the present study, the mouthwash containing hydrogen peroxide demonstrates one of the best clinical effectiveness with highest reduction of all indexes. Only 5 of the patients in the recent research reported burning sensation when using the above-mentioned mouthrinse. This corresponds to other studies stating that low percentage of hydrogen peroxide (<1.5%) do not lead to side effects.^[21] Furthermore, mouthwashes with hydrogen peroxide have been proposed to reduce the COVID 19 viral load, which leads to pandemic situation in the recent 2 years.^[22]

In the present study, we used clinically for the first time the combination of essential oils and chlorhexidine as active ingredients of mouthwash. All indexes tested reduced significantly after 21 days of its application in the adjunct treatment of gingivitis. The combination of essential oils and chlorhexidine seems to have better antimicrobial effectiveness when used alone, which could lead to better clinical effectiveness in gingivitis treatment as it is a plaque induced disease.^[1,23,24] However, 30% of the patients report

Table 5. Presence of side effects after mouthwash usage

		Presence of side effects				Total	
		No	Burning	Pinching	Both		
Mouthwash	1. Essential oils	Count	19	1	10	0	30
		%	63.3%	3.3%	33.3%	0.0%	100.0%
	2. Essential oils + 0.12% CHX	Count	19	6	5	0	30
		%	63.3%	20.0%	16.7%	0.0%	100.0%
	3. Placebo	Count	30	0	0	0	30
		%	100.0%	0.0%	0.0%	0.0%	100.0%
	4. 0.2% CHX	Count	30	0	0	0	30
		%	100.0%	0.0%	0.0%	0.0%	100.0%
	5. Prebiotic	Count	30	0	0	0	30
		%	100.0%	0.0%	0.0%	0.0%	100.0%
	6. 0.8% H ₂ O ₂	Count	25	2	2	1	30
		%	83.3%	6.7%	6.7%	3.3%	100.0%
Total	Count	153	9	17	1	180	
	%	85.0%	5.0%	9.4%	6%	100.0%	

burning sensation where this percentage is the same in the group treated only with essential oils.

Probiotics are used in mouthwashes demonstrating clinical effectiveness comparable to mouthwashes with chlorhexidine.^[12] There are no studies reporting the effectiveness of mouthwash containing prebiotic. Firstly, prebiotics are defined as “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”.^[12] Since that time they are widely used alone or in combination with probiotics in gastrointestinal diseases treatment.^[25] The oral microbiome is highly diverse with more than 700 species included, which could be divided into two basic groups – beneficial bacteria and pathogenic bacteria. The additional use of prebiotics could shift the composition of the dental biofilm from mostly pathogenic to beneficial species promoting oral health.^[26] The present study demonstrates promising results in using prebiotics in adjunct treatment of gingivitis – there is significant reduction of all parameters tested.

CONCLUSIONS

All tested mouthwashes demonstrated significant, but also varying degrees of improvement in clinical parameters after their use in adjunctive therapy of generalized gingivitis. New formulas with prebiotic and combination of essential oils and chlorhexidine indicate promising effectiveness.

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The study is registered in ClinicalTrials.gov under the following identifier: NCT04733196.

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Рандомизированное контролируемое исследование по сравнению клинической эффективности ополаскивателей для полости рта на основе эфирных масел, хлоргексидина, перекиси водорода и пребиотиков при лечении гингивита

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Резюме

Цель: Настоящее клиническое исследование было направлено на изучение клинической эффективности 5 видов ополаскивателей для полости рта на основе различных активных веществ.

Материалы и методы: В исследование включено 180 пациентов, разделённых на 6 групп по 30 человек, в каждой группе полоскание рта производилось одним из следующих видов ополаскивателей на основе: эфирных масел, комбинации эфирных масел и 0.12% хлоргексидина, перекиси водорода (0.8%), пребиотика, 0.2% хлоргексидина и плацебо. Всем участникам было проведено профессиональное механическое удаление зубного налёта, после чего им было рекомендовано полоскать рот 15 мл ополаскивателя 2 раза в день в течение 21 дня. В течение периода исследования за пациентами наблюдали в дни 0, 14 и 21, оценивая индекс гигиены полости рта, индекс дёсен, индекс кровоточивости и наличие побочных эффектов.

Результаты: Десневой индекс, индекс кровоточивости и индекс гигиены полости рта были статистически значимо снижены во всех группах лечения. Дополнительное использование жидкостей для полоскания рта продемонстрировало лучшую клиническую эффективность по сравнению с механическим контролем налёта (и жидкостью для полоскания рта плацебо). Десневой индекс и индекс зубного налёта были наиболее значительно снижены в группе, использующей жидкость для полоскания рта с перекисью водорода. Снижение индекса кровоточивости было наиболее значительным в группе, использовавшей 0.2% хлоргексидина.

Заключение: Все протестированные жидкости для полоскания рта продемонстрировали значительную клиническую эффективность в разной степени при лечении гингивита. Новые формулы с пребиотиком и комбинацией эфирных масел и хлоргексидина указывают на многообещающую эффективность.

Ключевые слова

хлоргексидин, эфирные масла, гингивит, перекись водорода, жидкости для полоскания рта, пребиотики