

A Pilot Study Into the Effectiveness of Two Antimicrobial Mouthrinses in a Group of Russian Adults With Gingivitis

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Abstract

Aim: To evaluate the effectiveness of antimicrobial mouthrinses with chlorhexidine and essential oils for the improvement of oral hygiene and the reduction of gingival inflammation in patients with gingivitis and to reveal their influence on oral microflora. **Methods:** 86 adult patients (aged 20-35 years old) with chronic marginal gingivitis were randomly selected in three groups. Test group 1 used mouthrinse *One Drop Only Ondrohexidine* (One Drop Only GmbH, Germany), containing chlorhexidine digluconate (0.1%) and potassium fluoride (250 ppm). Test group 2 used *Listerine Cool Mint* (McNeil PPC, USA), containing thymol (0.064%), eucalyptol (0.092%), methyl salicylate (0.06%), and menthol (0.042). The control group just rinsed the oral cavity with water. Values of the Patient Hygiene Performance Index (PHP), Approximal Plaque Index (API), Gingival Index (GI), and Sulcular Bleeding Index (SBI) were assessed at baseline and after two, four, and six weeks. Evaluation of pathogenic and resident bacterial species concentration in gingival sulcus biofilm (by cultural bacteriological examination) was conducted at baseline and after six weeks. **Results:** Oral hygiene (toothbrushing and six-week mouthrinse application) led to a significant decrease in plaque accumulation on smooth (by 58.1 and 62.3% in test groups 1 and 2, respectively) and approximal (by 53.9 and 58.5% in test groups 1 and 2, respectively) tooth surfaces, reduction of gingival inflammation (by 69.0 and 71.6% in test groups 1 and 2, respectively) and gingival sulcus bleeding (by 80.6 and 82.3% in test groups 1 and 2, respectively). Final indices values in both test groups were significantly ($P < 0.001$) lower compared to those at baseline and those after six weeks in the control group. A considerable decrease in the specific perio-pathogens that were assessed was observed, together with a significant reduction in their concentration. The antimicrobial ingredients of mouthrinses did not cause adverse changes in the normal microflora. **Conclusion:** Combined toothbrushing and antimicrobial mouthrinses application provided significant improvement of oral hygiene level and periodontal condition in patients with gingivitis.

Key Words: Gingivitis, Antibacterial Mouthrinses, Oral Hygiene Level, Oral Microflora

Introduction

At present, the prevalence of periodontal diseases in Russia is rather high and has a tendency to grow. The Russian National Oral Health Survey conducted in 2007-2008 revealed that 81% of 35-44-year-olds had signs of periodontal inflammation [1].

A similar situation has been observed in European countries and the USA. Recent surveys have shown that in Germany, only 20% of adults had healthy gums [2]. In Ireland, gingivitis was observed in 77% of 16-24-year-olds and in 91% of 55-64-year-olds [3]. About two million of

Americans younger than 35 years of age and another four million over this age required treatment for periodontal diseases [4].

Bacteria in dental plaque are one of the main factors causing periodontal inflammation [5]; therefore, careful plaque control is very important. However, mechanical plaque removal is inadequately performed by most members of the population. The need for additional help in controlling bacterial plaque provides the rationale for patients to use antimicrobial mouthrinses in addition to their mechanical oral hygiene regimens [6].

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A number of controlled clinical trials have demonstrated that rinsing with antiseptics (chlorhexidine, cetylpyridinium chloride, triclosan, and essential oils) reduces supragingival plaque and gingivitis [7-12]. It is also very important to study the influence of mouthrinses on the quality and quantity of the pathogenic and normal oral microflora. In one study, rinsing with chlorhexidine gluconate had the lowest minimal inhibitory concentration for the growth of most of the 40 test species (including periodontal and cariogenic pathogens) when compared with essential oil and herbal mouthrinses [13]. In spite of numerous investigations, there are still some questions when comparing the effectiveness of different types and concentrations of mouthrinses.

In patients with gingivitis, it is necessary, on one hand, to achieve a decrease in periodontal pathogens and, on the other, to prevent occurrence of adverse changes of resident microbial species.

Aim

The aim of this clinical and laboratory study was to evaluate the effectiveness of antimicrobial mouthrinses with chlorhexidine and essential oils to improve oral hygiene and to reduce gingival inflammation in patients with gingivitis, as well as to reveal their influence on the oral microflora.

Methods

The clinical investigation was carried out by three dentists in Preventive Dentistry Department of Moscow State University of Medicine and Dentistry. They had been previously trained and calibrated in periodontal assessment.

One hundred and five patients who met the inclusion criteria and who attended the dental clinic were examined during one month. The inclusion criteria were: age from 20-35 years of age, poor oral hygiene level, and signs of gingival inflammation corresponding with chronic marginal gingivitis. The criteria for exclusion were advanced periodontal inflammation, presence of fixed orthodontic devices, taking antibiotics or anti-inflammatory medicines less than one month before the study began, known hypersensitivity to the mouthrinses, pregnant females and nursing mothers. Eighty-six of those who met the inclusion criteria agreed to participate in this study and signed informed consent forms. Ethical approval for the study was given by the Ethical Committee of Moscow State University of Medicine and Dentistry.

Study design

Patients were randomly allocated into three groups (*Table 1*) that were similar in age, gender, oral hygiene and periodontal status. After baseline examination, all participants were instructed about the use of the mouthrinses and given oral hygiene instruction. All received professional tooth cleaning.

Methods of oral examination

Oral examination was performed at the beginning of the study (baseline), and after two, four, and six weeks. The following variables were assessed:

- Oral hygiene level using the Patient Hygiene Performance Index (PHP) (Podshadley & Haley 1968) [14].
- Dental plaque on approximal tooth surfaces using the Approximal Plaque Index (API) (Lange & Plagmann 1977) [15].

Table 1. Distribution of Subjects According to Oral Hygiene Regimen

Group	Number of participants	Oral hygiene regimen
Test 1	31	Toothbrushing ¹ for 3 minutes, mouthrinsing with water, and after 30 minutes with 10 ml of undiluted mouthrinse containing chlorhexidine (0.1%) ² for 30 seconds
Test 2	32	Toothbrushing ¹ for 3 minutes, mouthrinsing with water, and after 30 minutes with 15 ml of undiluted mouthrinse containing essential oils ³ for 30 seconds
Control	23	Toothbrushing ¹ for 3 minutes, rinsing mouth with water

¹ All subjects used identical toothbrushes with medium bristles and toothpastes "One Drop Only Concentrate" (One Drop Only GmbH, Germany), containing essential oils, fluorides (1450 ppm), calcium compounds, sodium bicarbonate, and silica.

² Alcohol-free mouthrinse "One Drop Only Ondrohexidine" (One Drop Only GmbH, Germany), containing chlorhexidine digluconate (0.1%), potassium fluoride (250 ppm), xylitol.

³ Mouthrinse "Listerine Cool Mint" (McNeil PPC, USA), containing thymol (0.064%), eucalyptol (0.092%), methyl salicylate (0.06%), menthol (0.042%). Alcohol content: 22.6%.

- Gingival inflammation degree using the Gingival Index (GI) (Löe & Silness 1963) [16].
- Gingival sulcus bleeding using the Sulcular Bleeding Index (SBI) (Muhleman 1968) [17].

Methods of microbiological investigation

In the microbiological study, 14 patients with the highest plaque index values at baseline (seven from test group 1 and seven from test group 2) were selected. The sampling was carried out before meals and toothbrushing during baseline examination and after six weeks. In each patient, one site in most inflamed area of the periodontium was chosen and cleaned with a sterile cotton-wool tampon. Supragingival plaque was removed using sterile curette and a sample of gingival sulcus biofilm was collected. Then an absorbent paper point (standard no. 30) was placed in the gingival sulcus, excluding contact with saliva and tooth enamel. Biofilm samples were placed in test tubes, containing 500 ml of Aim's transport medium and delivered to microbiological laboratory.

Evaluation of the counts of microbial pathogenic and resident species was carried out by cultural bacteriological examination using anaerobic cultivation technique.

Statistical analysis

Study data were entered into statistical software (Statistica 6.0 for Windows; Statsoft Inc., Tulsa, OK, USA) and Student's t-test was used to compare the difference between groups. Results were tested for significance at the $P < 0.05$ level.

Results

Evaluation of oral hygiene level

The baseline examination revealed that the oral hygiene level of all participants was unsatisfactory

according to PHP and API indices criteria. Changes in plaque indices values are shown in *Figures 1* and *2*. Differences in baseline plaque indices values between both test and control group were not significant ($P > 0.05$) (*Table 2*).

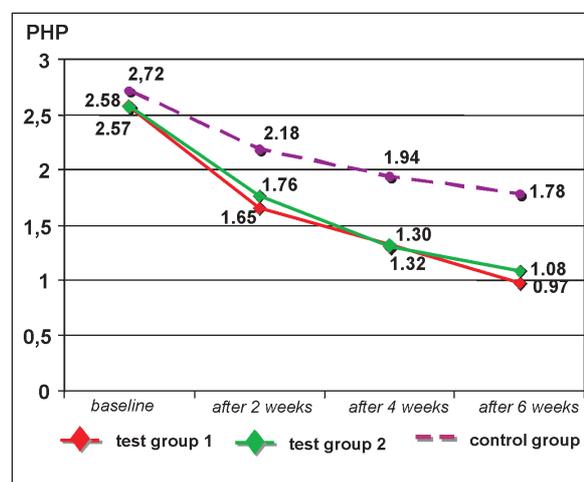


Figure 1. Patient Hygiene Performance Index values changes over 6 weeks.

After two weeks, significant reduction of dental plaque on smooth and approximal tooth surfaces compared to baseline was observed in all groups ($P < 0.001$). However, in the control group mean values of plaque indices were significantly higher than in the test groups. The amount of plaque on approximal tooth surfaces was lower in test group 1 than in test group 2 ($P < 0.05$).

After four weeks, in both test groups the mean values of the PHP and API indices were significantly lower compared to previous examination ($P < 0.01$ and $P < 0.001$ respectively) and to the control group ($P < 0.001$). At the same time, differences between test groups were non-significant ($P > 0.05$).

At the final examination, significant reduction of dental plaque compared with previous data was

Table 2. Changes of Plaque Indices Values ($M \pm m$) Over 6 Weeks

Plaque Indices	Group	Baseline (1)*	2 weeks (2)*	P (1-2)	4 weeks (3)*	P (2-3)	6 weeks (4)*	P (3-4)	Plaque reduction (%) after 6 weeks
PHP	Test 1	2.57±0.08	1.65±0.10	<0.001	1.32±0.08	<0.01	0.97±0.09	<0.01	62.3%
	Test 2	2.58±0.11	1.76±0.11		1.30±0.09		1.08±0.08		>0.05
	Control	2.72±0.14	2.18±0.10	<0.01	1.94±0.09	>0.05	1.78±0.08	34.6%	
API	Test 1	65.4±2.1	40.8±1.3	<0.001	32.5±1.3	<0.001	27.1±1.2	<0.01	58.5%
	Test 2	63.6±1.9	44.9±1.4		32.6±1.5		29.3±1.4		>0.05
	Control	61.3±2.2	49.7±1.8		48.2±1.9	>0.05	46.6±1.7	24.0%	

* Number of examination

observed in test group 1 ($P<0.01$). Thus, after six weeks of antimicrobial mouthrinses use, plaque accumulation had decreased on both smooth (by 62.3% and 58.1% in test groups 1 and 2, respectively) and approximal surfaces (by 58.5% and 53.9% in test groups 1 and 2, respectively). The mouthrinse containing chlorhexidine was more effective than the one containing essential oils but the difference was not significant ($P>0.05$).

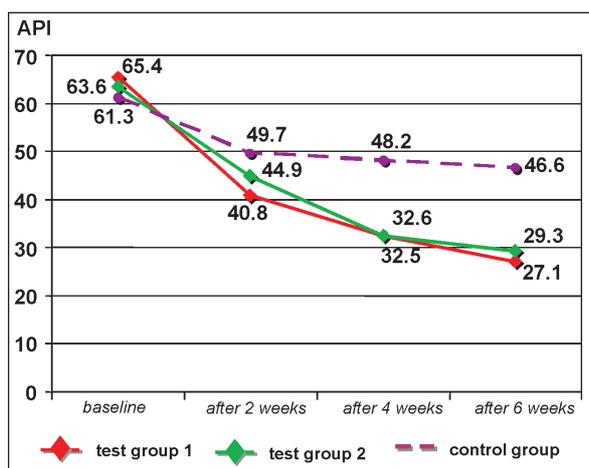


Figure 2. Approximal Plaque Index values changes over 6 weeks.

In the control group, where subjects performed oral hygiene care without antimicrobial mouthrinses, reduction of dental plaque on smooth and approximal tooth surfaces was 34.6% and 24.0%, respectively. Mean final values of PHP and API indices were significantly higher than in test groups ($P<0.001$).

As a side effect, many of the subjects in test group 1 had yellow-brown colouring of the teeth (61.3%) and the tongue (32.3%).

Evaluation of periodontal status

At baseline all subjects had signs of gingival inflammation: bleeding on probing, hyperaemia and swelling of the gingival margin. Mean values for the GI in both test and control groups indicated moderate gingivitis. Changes in GI values are shown in *Figure 3* and in the SBI in *Figure 4*.

There were no significant differences between groups in values of the GI and SBI indices at baseline. After two weeks, considerable improvement of periodontal condition was observed in all groups (*Table 3*). It was characterised by significant decrease in the degree of gingival inflammation and a reduction of gingival sulcus bleeding as compared with baseline data ($P<0.001$). The most anti-

inflammatory effect had been achieved in test groups, where mean values of GI and SBI indices were significantly lower than those of the control group ($P<0.001$).

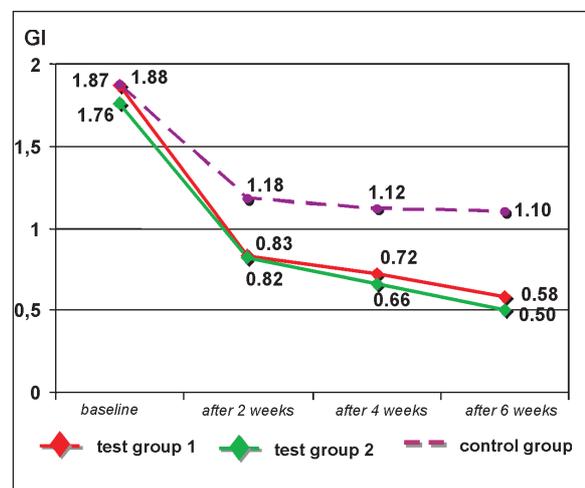


Figure 3. Gingival Index values changes over 6 weeks.

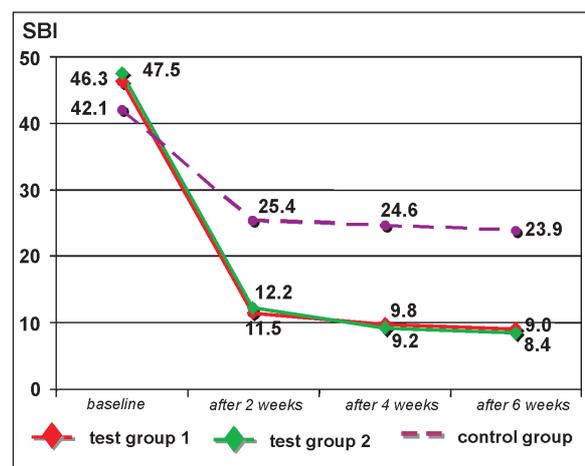


Figure 4. Sulcular Bleeding Index values changes over 6 weeks.

At the next oral examination, there were no significant changes in periodontal indices (GI and SBI) values except in test group 2, where a decrease in the GI value ($P<0.05$) was observed between four and six weeks. Differences between test groups were non-significant. After six weeks, the reduction in gingival inflammation (as assessed by GI) was 69.0% in test group 1, 71.6% in test group 2, and 41.5% in the control group. Mean values of the SBI decreased by 80.6%, 82.3%, and 43.2%, respectively. Thus both test mouthrinses had an almost identical anti-inflammatory effect, which was considerably better than in control group.

Table 3. Changes of Periodontal Indices Values (M+m) Over 6 Weeks”

Periodontal Indices	Group	Baseline (1)*	2 weeks (2)*	P (1-2)	4 weeks (3)*	P (2-3)	6 weeks (4)*	P (3-4)	Reduction of gingival inflammation and sulcus bleeding (%)
GI	Test 1	1.87±0.12	0.83±0.09	<0.001	0.72±0.07	>0.05	0.58±0.05	>0.05	69.0%
	Test 2	1.76±0.09	0.82±0.07		0.66±0.05		0.50±0.04	<0.05	71.6%
	Control	1.88±0.12	1.18±0.07		1.12±0.09		1.10±0.10	>0.05	41.5%
SBI	Test 1	46.3±3.3	11.5±2.1	<0.001	9.8±1.2	>0.05	9.0±1.3	>0.05	80.6%
	Test 2	47.5±2.6	12.2±1.6		9.2±1.2		8.4±1.0		82.3%
	Control	42.1±3.5	25.4±2.7		24.6±2.6		23.9±2.2		43.2%

* Number of examination

Results of microbiological study

At baseline, perio-pathogenic bacteria in different combinations were detected in all biofilm samples from patients with gingivitis (Figure 5). Each patient had from two to five of the assessed species in high concentrations (10⁵-10⁷ CFU).

The most frequently detected species were: *Tannerella forsythia* (100% in test group 1 and 72% in test group 2), *Aggregatibacter actinomycetemcomitans* (57% in both groups),

Streptococcus intermedius (43% and 72% in test groups 1 and 2, respectively), *Treponema denticola* (100% in test group 1) and *Prevotella intermedia* (43% in test group 2).

After six weeks of antimicrobial mouthrinsing, significant reduction of perio-pathogenic microorganisms in the gingival sulcus biofilm was observed. The frequency of *Aggregatibacter actinomycetemcomitans* detection decreased by 43% in both groups. The species that were more suscepti-

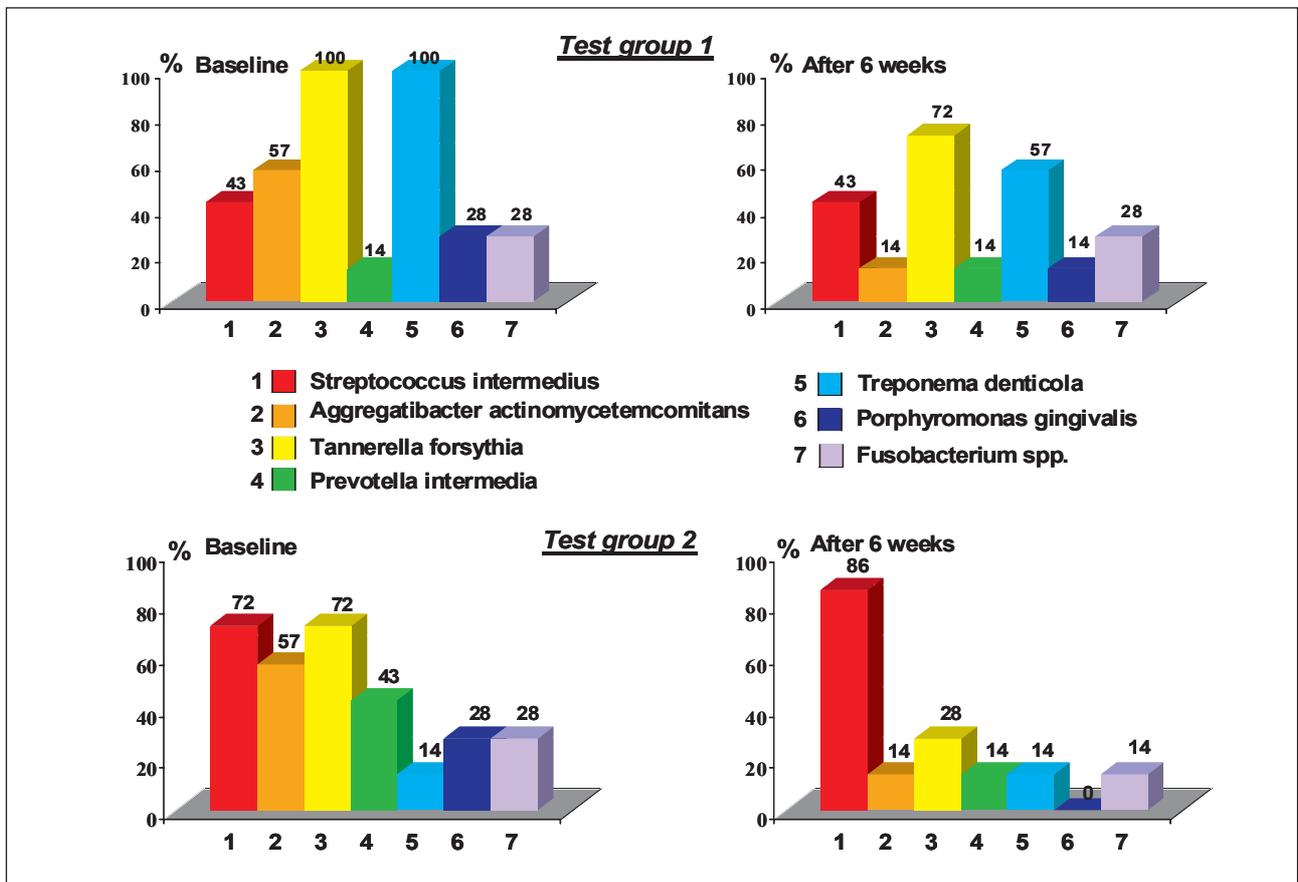


Figure 5. Changes in perio-pathogens frequency after antimicrobial mouthrinse application.

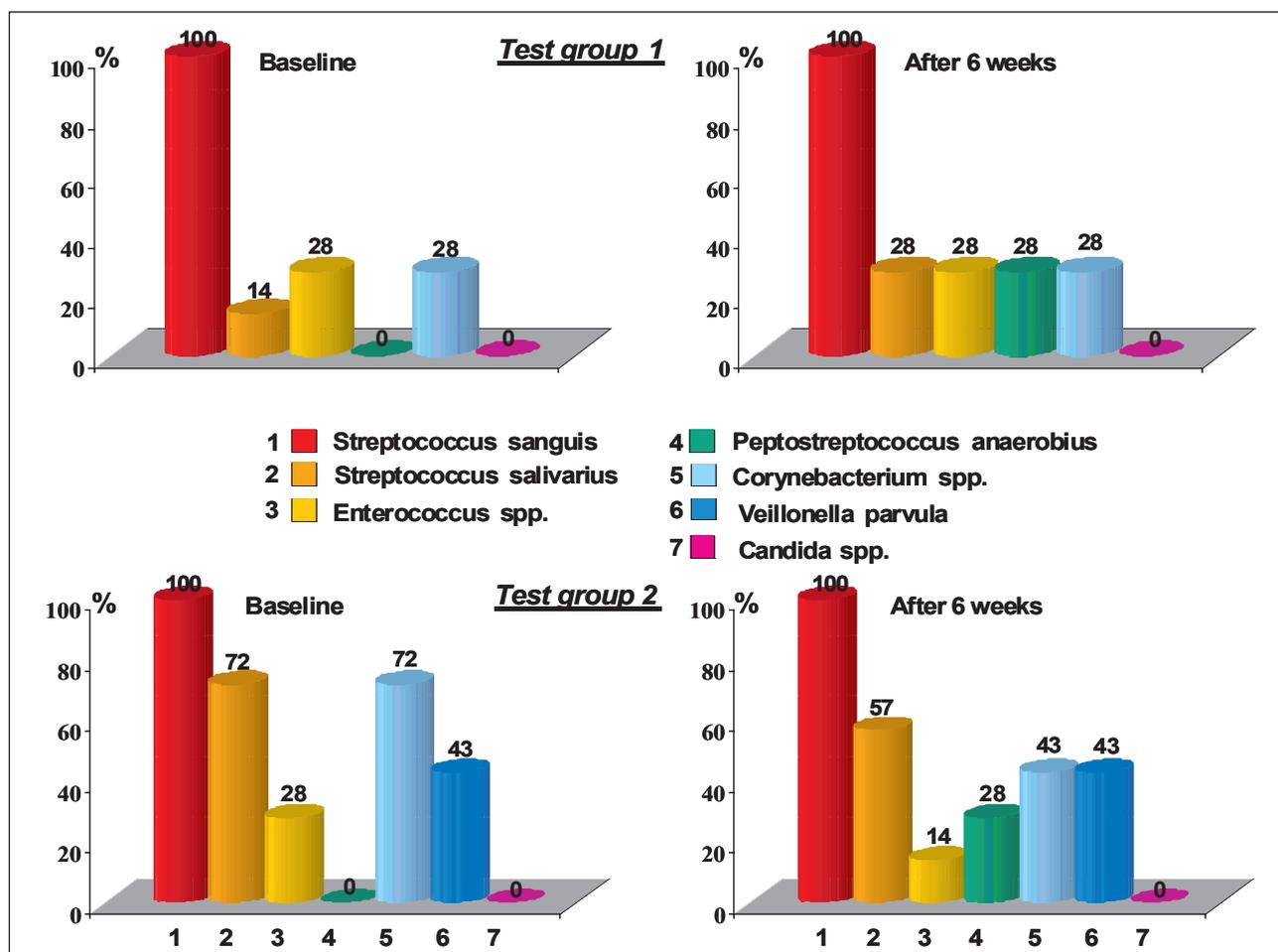


Figure 6. Changes of normal microflora composition after antimicrobial mouthrinse application.

Table 4. Changes of Perio-Pathogens Concentration After Antimicrobial Mouthrinse Application

Perio-pathogens specimens	Test group 1	Test group 2
<i>Streptococcus intermedius</i>	>0.05	>0.05
<i>A. actinomycetemcomitans</i>	<0.05	<0.05
<i>Bacteroides forsythus</i>	<0.05	>0.05
<i>Prevotella intermedia</i>	-	<0.05
<i>Treponema denticola</i>	>0.05	-
<i>Porphyromonas gingivalis</i>	<0.05	<0.05
<i>Fusobacterium spp.</i>	<0.05	<0.05

Legend:

- Increase
- Decrease
- -

ble to chlorhexidine appeared to be *Treponema denticola* and *Tannerella forsythia* (which saw a reduction by 43% and 28%, respectively) and to essential oils — *Tannerella forsythia*, *Prevotella intermedia* and *Porphyromonas gingivalis*. *Streptococcus intermedius* was resistant to antimi-

crobial action of both mouthrinses. Concentration of most perio-pathogens significantly decreased (by two to three times) compared with baseline levels, except for *Prevotella intermedia* (in test group 1) and *Streptococcus intermedius* (in both test groups) (Table 4).

Use of antimicrobial mouthrinses during six weeks did not cause adverse changes in the normal microflora. Its composition was stable, some resident specimens (*Peptostreptococcus anaerobius*) were recovered, and no cases of *Candida spp.* were detected (Figure 6).

Discussion

Very few studies into the effectiveness of mouthrinses against plaque and gingival inflammation have previously been performed in Russia. The results of the clinical study demonstrated that both of the tested mouthrinses containing chlorhexidine and essential oils were effective and led to an improvement in oral hygiene and in the periodontal status (as assessed using the GI and SBI) in patients with gingivitis.

The use of antimicrobial mouthrinse led to a considerable reduction in dental plaque accumulation both on smooth and approximal tooth surfaces. Final values of plaque indices in test groups were significantly lower compared to baseline data ($P < 0.001$) and to corresponding values in the control group. Application of antimicrobial mouthrinses after toothbrushing increased the effectiveness of plaque control, especially in remote interproximal sites. Probably, active ingredients of the mouthrinse penetrate the biofilm and prevent plaque accumulation [18].

A systematic review of six-month randomised clinical studies had shown that most of them (21) supported the efficacy of mouthrinses with essential oils and a smaller body (seven) the effect of mouthrinses with 0.12% chlorhexidine [12]. Results of other clinical studies confirm the long-term plaque- and gingival bleeding-reduction properties of mouthrinses containing chlorhexidine and essential oils [9,23,24].

In our study, mouthrinsing with chlorhexidine was more effective for the reduction of dental plaque than with the essential oils mouthrinse; however, differences were non-significant. This may have been due to chlorhexidine's affinity for various oral surfaces. Its cationic molecule binds to the anionic compounds of salivary glycoproteins and is then released for eight to 12 hours in an active form [19], reducing absorption to the tooth surface needed for the formation of the dental pellicle and bacterial colonisation [20].

Use of an oral hygiene regimen including toothbrushing and antimicrobial mouthrinsing increased the effectiveness of cleaning both at smooth (23.6% and 27.7% in test groups 1 and 2,

respectively) and at approximal tooth surfaces (30.0% and 34.6% in test groups 1 and 2, respectively) compared with toothbrushing alone. It has also been reported as more effective than toothbrushing combined with flossing in the control of approximal plaque and gingivitis [21,22].

Signs of gingival inflammation reduced significantly in both test groups compared to the control group. Use of antimicrobial mouthrinses in addition to toothbrushing provided an increase in anti-inflammatory effectiveness, as measured by the GI (by 27.5% and 30.1% in test groups 1 and 2, respectively) and a gingival bleeding reduction, as measured by the SBI (by 37.3% and 39.1% in test groups 1 and 2, respectively).

In our study, there were no significant differences of GI and SBI indices values between test groups after six weeks, indicating equivalence of tested mouthrinses in improving gingival health. A similar result has been reported in a recent study [25].

The most common side-effect of chlorhexidine is the formation of extrinsic yellow-brown stains on the teeth and tongue [26]. In our study, this was observed in most (61.3%) subjects in test group 1, who used a chlorhexidine-containing mouthrinse.

Probably, the cause of stains was due to a reaction between the absorbed chlorhexidine and the chromogens present in food and drink (tea, coffee, soft drinks) including polyphenols [27]. So in order to prevent staining, after use of mouthrinse with chlorhexidine it is necessary to refrain from consuming these products or smoking for one hour.

Results of microbiological study confirm antimicrobial effectiveness of the tested mouthrinses. After six weeks, a significant reduction in periodontal pathogens and their concentration was observed. It is well known that chlorhexidine possesses high bactericidal activity against gram-positive and gram-negative microorganisms and yeast cells. It has a strong affinity for the cell wall of microorganisms and changes their surface structures. As a result, osmotic equilibrium is lost and cytoplasmic membrane extrudes, forming vesicles and precipitating the cytoplasm. These precipitations inhibit the repair of the cell wall and bacteria are no longer able to recover [28]. Active ingredients of the other mouthrinse—phenolic essential oils (thymol and eucalyptol)—destroy the cell wall, inhibit bacterial products, and also endotoxin from gram-negative bacteria [8]. Results of an *in vitro* study have confirmed the bactericidal effect; most bacteria in saliva and plaque died after a 30-second exposure to an essential oils mouthrinse [29].

Application of antimicrobial mouthrinses during six weeks did not cause adverse changes in the normal microflora. Results of long-term studies [30,31] have also revealed no dysbacteriosis or microbial resistance after 3-6 months of using the mouthrinses that were tested in this study. The authors consider that regular use of an antimicrobial mouthrinse should especially be recommended to patients with insufficient mechanical plaque removal. In our opinion, it is very important to try to improve patients' oral hygiene skills during each visit to a dentist or dental hygienist. Then the effectiveness of oral disease prevention and treatment will be much improved.

Conclusions

- Regular application of antimicrobial mouthrinses containing chlorhexidine

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