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Koroleva, Irina V; Mikhaylova, Ekaterina S; Zhukovskaia, Ekaterina S.; Kraeva, Lyudmila A.; Suvorov, Alexander N.

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Clinical and microbiological evaluation of the efficacy of autoprobiotics in the combination treatment of chronic generalized periodontitis

Evaluación clínica y microbiológica de la eficacia de los autoprobióticos en el tratamiento combinado de la periodontitis crónica generalizada.

Irina V Koroleva
"Institute of Experimental Medicine», St. Petersburg,
Russia, Rusia
ivkoroleva@yandex.ru

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Ekaterina S Mikhaylova
Saint Petersburg State University, St. Petersburg, Russia,,
Rusia
catpara72@mail.ru

Ekaterina S. Zhukovskaia
Kirov Military Medical Academy, St. Petersburg, Russia,
Rusia
kateri241296@gmail.com

Lyudmila A. Kraeva
Saint Petersburg Pasteur Institute, St. Petersburg, Russia,
Rusia
lykraeva@yandex.ru

Alexander N. Suvorov
Federal State Budgetary Scientific Institution "Institute of
Experimental Medicine», St. Petersburg, Russia, Rusia
alexander_suvorov1@hotmail.com

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ABSTRACT:

Combination treatment of patients with inflammatory periodontal diseases may be ineffective due to the variability of periodontal pathogens and the polyetiology of the disease. This disadvantage can be overcome by using highly antagonistic, enzymatic, and immunostimulating drugs, in addition to the main treatment. The objective of this study is to evaluate the efficacy of autoprobiotics clinically and microbiologically in the combination treatment of chronic generalized periodontitis. The presented study involved a survey of 37 patients aged 29 to 64 years with mild chronic generalized periodontitis. The patients were divided into three groups. Group I consisted of patients whose combination treatment included an *S. salivarius*-based autoprobiotic (subgroup 1 - patients who had their periodontal pockets irrigated with an autoprobiotic, subgroup 2 - patients who used oral baths with autoprobiotic). Group II consisted of patients who used a common *S. salivarius*-based probiotic in combination treatment (subgroup 1 - patients who had their periodontal pockets irrigated with a probiotic, subgroup 2 - patients who used oral baths with a probiotic). The control group consisted of patients with mild chronic generalized periodontitis, whose combination treatment consisted of professional oral hygiene and correction of individual hygiene. Microbiological examination of the content of periodontal pockets was carried out using PCR screening for periodontal pathogens, such as *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*. Based on the clinical and microbiological results of the study, the efficacy of an autoprobiotic and probiotic based on *S. salivarius* in the combination treatment of mild chronic generalized periodontitis was demonstrated.

KEYWORDS: chronic periodontitis, periodontal pathogens, microbiota, probiotics, autoprobiotics, probiotic therapy.

RESUMEN:

El tratamiento combinado de pacientes con enfermedades periodontales inflamatorias puede resultar ineficaz debido a la variabilidad de los patógenos periodontales y la polietilogía de la enfermedad. Esta desventaja puede superarse mediante el uso de fármacos altamente antagonistas, enzimáticos e inmunoestimulantes, además del tratamiento principal. El objetivo de este estudio es evaluar clínica y microbiológicamente la eficacia de los autoprobióticos en el tratamiento combinado de la periodontitis crónica generalizada.

El estudio presentado incluyó una encuesta de 37 pacientes de 29 a 64 años con periodontitis crónica generalizada leve. Los pacientes se dividieron en tres grupos. El grupo I consistió en pacientes cuyo tratamiento combinado incluía un autoprobiótico basado en *S. salivarius* (subgrupo 1 - pacientes que tenían sus bolsas periodontales irrigadas con un autoprobiótico, subgrupo 2 - pacientes que usaron baños orales con autoprobiótico). El grupo II consistió en pacientes que usaron un probiótico común a base de *S. salivarius* en tratamiento combinado (subgrupo 1 - pacientes que tenían sus bolsas periodontales irrigadas con un probiótico, subgrupo 2 - pacientes que usaron baños orales con un probiótico). El grupo control estuvo formado por pacientes con periodontitis crónica generalizada leve, cuyo tratamiento combinado consistió en higiene bucal profesional y corrección de la higiene individual. El examen microbiológico del contenido de las bolsas periodontales se llevó a cabo mediante el cribado por PCR de patógenos periodontales, como *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*. Con base en los resultados clínicos y microbiológicos del estudio, se demostró la eficacia de un autoprobiótico y un probiótico a base de *S. salivarius* en el tratamiento combinado de la periodontitis crónica generalizada leve.

PALABRAS CLAVE: periodontitis crónica, patógenos periodontales, microbiota, probióticos, autoprobióticos, terapia probiótica.

INTRODUCTION

Periodontitis is a polyetiological disease. Its onset, course, and treatment depend on many endo- and exogenous factors, some of which are the subject of modulation. However, the leading role in the development of periodontitis is played by microbiological and immunological changes in the patient's body against the background of genetic predisposition¹. Inflammatory periodontal diseases are caused by a mixed microbiota, a combination of different types of microorganisms, which can vary in patients depending on the severity of periodontitis and the localization of the lesion¹⁻³.

Based on the data on the ecological interactions of microorganisms in periodontal pockets, as well as on a qualitative analysis of samples of subgingival dental deposits, microorganisms found in the periodontal pockets of patients were in the composition of "yellow", "purple", "green", "orange", "red" complexes and 3 microorganisms of an extra-complex organization^{4,5}. The study of the ecological relationships within the biofilm and dental plaque, as well as the study of the stages of colonization of the tooth surface, indicates that the "yellow", "purple" and "green" complexes are conditionally pathogenic microflora, as they are involved in the process of early colonization, while in the absence of "red" and "orange" complexes they do not show pathogenic properties in relation to the periodontal attachment^{4,5}.

The efficacy of combination treatment of patients with inflammatory periodontal diseases, including local and general antibiotic therapy, is ambiguous due to the variability of periodontitis pathogens, the difficulty of predicting the course of the inflammatory process in the periodontal tissues, and the polyetiology of the disease. Conservative drug treatment of inflammatory periodontal diseases is aimed at eliminating periodontal pathogens and factors contributing to the colonization of periodontal structures by periodontopathogens, relieving symptoms of inflammation, regenerating periodontal tissues, and increasing the body's reactivity. Nevertheless, the microbiota of periodontal pockets has a different sensitivity and can be resistant to the antibacterial drugs used, which affects the efficacy of the treatment and is manifested by the progression of the inflammatory process in the periodontal tissues⁶.

The emergence of new antibiotic-resistant strains of microorganisms, the widespread of viral and fungal diseases, and the increasing allergization of the population have given rise to the interest in bacterial drugs (probiotics), which include live microorganisms - representatives, as a rule, of obligate human microflora, which when ingested in a sufficient amount into the body, retain their activity and vitality, have a positive

effect on the patient's health.. Various types of bifidobacteria are used as probiotics (*B. longum*, *B. breve*, *B. infantis*, *B. bifidum*, *B. adolescentis*, *B. animalis*), lactobacilli (*L. rhamnosus*, *L. acidophilus*, *L. casei*, *L. bulgaricus*, *L. gasseri*) and other microorganisms (*L. cremoris*, *L. lactis*, *S. thermophilus*, *Enterococcus faecium*, *Saccharomyces boulardi*). Due to their great genetic diversity, they differ from each other in their properties, which explains the discrepancies in the results of assessing their efficacy⁸⁻¹¹.

According to modern concepts, probiotic microorganisms have multidirectional "direct" and "indirect" effects; they affect not only the microflora of the mucous membranes but also the epithelium and the immune system. The direct probiotic effect disrupts the mechanisms of plaque formation and affects antimicrobial compounds of probiotics on microorganisms. Such compounds include organic acids, hydrogen peroxide, and peptides that form lactic acid bacteria. Strengthening the work of the body's defense systems is the basis of an indirect probiotic effect. It has been proven that the interaction of lactic acid bacteria with macrophages and T-lymphocytes leads to an increase in the synthesis of cytokines¹². Thus, the mechanisms of action of probiotics include competitive exclusion, suppression of the growth of pathogenic and opportunistic microorganisms, and immune modulation.

Antagonism of probiotics with periodontopathogens, aggregation with oral bacteria, and interaction with oral epithelium have been noted⁹. Antagonism with periodontal pathogens and aggregation with oral bacteria leads to a decrease in the pathogenicity and cariogenic potential of microorganisms in the biofilm¹³, as well as to a decrease in the potential load of pathogens in the oral biofilm¹⁴. Interacting with the epithelium of the oral cavity, probiotics can enhance the function of the epithelial barrier and activate immune responses^{15,16}.

Modern research is aimed at studying the effectiveness of the use of probiotics both as monotherapy and in the combination treatment of inflammatory periodontal diseases. Teughels et al. believe that the use of a probiotic as the only drug in the treatment of periodontitis has a weak effect¹⁷. Nevertheless, most studies have confirmed the efficacy of probiotics in the combination treatment of periodontitis. Penala et al. consider proven the effectiveness of the topical application of probiotics with *L. salivarius* and *L. reuteri* in the form of oral rinsing solutions, since three months after therapy an improvement in the state of periodontal tissues and relief of symptoms of inflammation was noted¹¹.

Modern research is aimed at creating probiotics based on *S. salivarius* and studying their effect on the state of tissues and organs of the oral cavity. *S. salivarius* is an oral streptococcus that forms the basis of the normal oral microbiota, which does not adversely affect the human body. The increased interest in the probiotic potential of *S. salivarius* is associated with the fact that some strains of this bacterial species produce a diverse set of bacteriocins, exozymes, dextranase, and urease, the activity of which can limit the progression of dental caries, reduce the accumulation of dental plaque, and increase its pH¹⁸. A modern direction in probiotic combination therapy is the assessment of the efficacy of autoprobiotics obtained based on autologous strains of microorganisms^{19,20}. The rationale for this line of research is the personalization of probiotic drugs without the risk of rejection by the body due to high histocompatibility²¹. Probiotics can have an unpredictable effect on the resident microflora: their interaction can be based on biocompatibility or probiotic versus host and host versus probiotic antagonism²².

The concept of using autoprobiotics in the combination treatment of inflammatory periodontal diseases is promising and requires further research to create drugs and assess their effectiveness. Existing studies prove the positive effect of autologous microorganisms on periodontal tissue. Suvorov et al. proved the prophylactic efficacy of autoprobiotics in the treatment of infectious and inflammatory diseases of the oral cavity and pharynx¹².

Thus, the high frequency and prevalence of periodontal diseases, the need for a personalized approach to the choice of a probiotic drug for the beneficial effect on periodontopathogens, the prospects of research

on the creation and assessment of the efficacy of autoprobiotics in the treatment of periodontal diseases determine the relevance and objective of the present investigation.

MATERIALS AND METHODS

Study subjects and clinical examination

We examined 37 patients (19 women and 18 men) aged 29 to 64 years (average age - 44.3 ± 1.5 years) diagnosed with mild chronic generalized periodontitis (Table 1).

Criteria for inclusion of patients in the study were reliable diagnosis of chronic generalized periodontitis and informed consent of the patient. Criteria for exclusion of patients from the study were smoking; any orthodontic appliances installed; severe concomitant subcompensated or decompensated pathology of internal organs, diabetes mellitus, benign or malignant tumors of any localization and etiology; HIV infection and other immunodeficiencies, active tuberculosis; the refusal of the patient from the examination.

We used clinical, radiological, and microbiological examination methods. Clinical examination of patients included taking the history of life and disease, clinical assessment of the state of periodontal tissues, determination of the Oral *Hygiene Index* (OHI-S) (Green, Vermillion, 1964), *Periodontal Index* (PI), the state of gingival inflammation by Parma's papillae-gum margin-alveolar (PMA index), Bleeding on probing (BOP) index (Amino, Bay, 1975) and Community periodontal index of treatment needs (CPITN) index (WHO, 1982). X-ray examination was carried out using a Galileos cone-beam computed tomography (Sirona, Germany).

Experiment design

The patients were divided into three groups. Group I consisted of patients whose combination treatment included an *S. salivarius*-based autoprobiotic (subgroup 1 - patients who had their periodontal pockets irrigated with an autoprobiotic, subgroup 2 - patients who used oral baths with autoprobiotic). Group II consisted of patients who used a common *S. salivarius*-based probiotic in combination treatment (subgroup 1 - patients who had their periodontal pockets irrigated with a probiotic, subgroup 2 - patients who used oral baths with a probiotic). The control group consisted of patients with mild chronic generalized periodontitis, whose combination treatment consisted of professional oral hygiene and correction of individual hygiene.

Clinical examination, treatment, and material sampling in patients of groups I and II were carried out according to a specific scheme, including 5 visits:

- 1) initial examination of the patient with a sampling of material from periodontal pockets and buccal mucosa for the microorganism's cultivation to create an *S. salivarius*-based autoprobiotic;
- 2) 4-5 days after the initial visit, professional oral hygienic treatment, correction of individual oral hygiene, and either autoprobiotic or probiotic therapy were performed;
- 3) 3-4 days after the second visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets, and either autoprobiotic or probiotic therapy were performed;
- 4) 6-7 days after the third visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets were performed;
- 5) 27-28 days after the fourth visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets were performed (Table 1).

Clinical examination, treatment, and material sampling in patients of the control group were carried out in 5 steps:

- 1) initial examination of the patient with a sampling of material from periodontal pockets;
- 2) 4-5 days after the initial visit, professional oral hygienic treatment, and correction of individual oral hygiene were performed;
- 3) 3-4 days after the second visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets were performed;

4) 6-7 days after the third visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets were performed;

5) 27-28 days after the fourth visit, a repeated examination of the patient's oral cavity, sampling of material from periodontal pockets were performed (Table 1).

TABLE 1
Research materials

Sample type	Sample description	Sample type	Sample description	Sample type	Sample description	Sample type	Sample description
Salivary	Salivary samples collected from the patient's oral cavity during the examination of the oral cavity	Periodontal	Periodontal samples collected from the patient's oral cavity during the examination of the oral cavity	Periodontal	Periodontal samples collected from the patient's oral cavity during the examination of the oral cavity	Periodontal	Periodontal samples collected from the patient's oral cavity during the examination of the oral cavity

Microbiota study

For microbiological and genetic studies, the samples were taken from periodontal pockets of each patient using sterile Absorbent Paper Points, Euronda (size 25) for 15 seconds. The resulting material was placed in a sterile Eppendorf tube, which was stored at -20°C until further PCR-test.

Isolation of total DNA was performed using the Express-DNA-Bio kit according to the instructions for use.

Using the Primer 3 and OLIGO 4.0 programs, oligonucleotide primers were created with the determination of their melting temperatures (Table 2).

DNA electrophoresis was carried out in a 1.0% agarose gel in a Hoefer HE 33 horizontal apparatus (Pharmacia, Sweden) using a TAE buffer for 30 minutes at 100V. To calculate the molecular weights of DNA fragments, a 100 bp Plus DNA ladder, DNA marker, was used.

TABLE 2
Oligonucleotide primers *P. gingivalis* *P. intermedia* *T. forsythia* *T. denticola* *S. salivarius*

Name	5' → 3'	T anneal	Fragment size (bps)
<i>P. gingivalis</i>			
Gin1	GTATATGCTCGACGAGGTGGAA	57.0	334
Gin2	ATTGTCCAGGGTAACTTCTTCG		
<i>P. intermedia</i>			
Int 1	AATACAGCCTTCGAGGGTTT	55.0	335
Int 2	TTCCGGTCAAGACAGTAGGGA		
<i>T. forsythia</i>			
For1	CGAGGGTTCAATACGCTGTT	54.0	572
For2	ATAAAAATCGCATCGCAAGG		
<i>T. denticola</i>			
Den1	TAATACCGAATGTGCTCATTTACAT	59.0	311
Den2	TCAAAGAAGCATTCCCTCTTCTTCTTA		

Autoprobiotic strains

The basis of an autoprobiotic or probiotic for the combination treatment of periodontitis was *S. salivarius*. Before the start of autoprobiotic therapy, the material was taken from the patient's cheek mucosa.

The cultivation of facultative anaerobes was carried out on a 2.5% Todd-Hewitt broth (THB) dense medium (Difco, USA) with the addition of 0.5% yeast extract (Helicon, Russia) and 5% ram blood at 37°C and 5% CO₂ for 18 hours.

A pure culture of *S. salivarius* was isolated from the biological material, monitoring the identification with MALDI TOF and PCR screening. The isolated cultures were stored in a liquid THB medium with 0.5% yeast extract with the addition of glycerol at -70°C.

To prepare an autoprobiotic, isolated culture of *S. salivarius* from a particular patient was grown on 14 mL of THB enriched with 0.5% yeast extract at 37°C for 18 hours. The resulting bacterial suspension was centrifuged. Then the precipitated cells were washed once with saline and suspended in the same solution to the initial volume.

The concentration of the bacterial suspension (C) was controlled by colony-forming unit per millilitre (CFU/mL) and photometrically. We used autoprobiotic or probiotic preparations with C from 4×10^8 to 6×10^8 CFU/mL. To create a general probiotic, the *S. salivarius* strain was used, which was previously isolated from the cheek mucosa of a healthy patient.

To monitor the colonization of *S. salivarius* during auto- and probiotic therapy, biological material was taken from the periodontal pockets of patients with subsequent inoculation on a solid nutrient medium, identification, and calculation of CFU/mL in the initial inoculation. All actions were carried out similarly to those described above.

Statistical analysis

All the data obtained were systematized in the Statistic program. The statistical significance of the differences and the reliability of the differences in indicators were determined using the Student's t-test. P values were considered statistically significant at $p < 0.05$. Plus, One-way ANOVA was used to evaluate the difference between groups.

RESULTS

Before treatment, all patients complained of bleeding while brushing their teeth, swelling, and inflammation of the gums. Examination of the oral cavity in all patients before treatment revealed exudation from the periodontal pockets, hyperemia of the marginal, attached gums. The loss of clinical periodontal attachment averaged 2.96 ± 0.03 mm.

The indicators of hygiene indices, bleeding and the state of periodontal tissues are presented in Table 3. The average value of the OHI-S index of patients was 3.72 ± 0.19 , which corresponds to a poor level of oral hygiene (> 3.1). The poor level of oral hygiene of patients is confirmed by the PI index, a high average value of which (1.73 ± 0.09) proves the presence of dental plaque in patients. The CPITN index value of 2.27 ± 0.07 shows the patients' need for professional oral hygiene and local anti-inflammatory therapy. Inflammation of the gums and papilla can be assessed not only visually, but also analyzed using the PMA index. The PMA index value of $43.3 \pm 1.8\%$ indicates the presence of moderate gingivitis in patients. Based on the high value of the BOP index ($70.3 \pm 2.4\%$), it can be concluded that there is a high degree of bleeding in the examined patients.

TABLE 3
The indicators of hygiene indices bleeding and the state of periodontal tissues in patients I II and control groups before treatment

Index	Group 1, subgroup 1	Group I, subgroup 2	Group II, subgroup 1	Group II, subgroup 2	Control group	Overall indicator
OHI-S	4.13 ± 0.50	3.19 ± 0.32	4.06 ± 0.40	3.80 ± 0.57	3.53 ± 0.39	3.72 ± 0.19
PI	1.88 ± 0.28	1.59 ± 0.19	1.80 ± 0.22	1.70 ± 0.31	1.69 ± 0.18	1.73 ± 0.09
CPITN	2.19 ± 0.17	2.31 ± 0.14	2.50 ± 0.10	2.19 ± 0.17	2.17 ± 0.15	2.27 ± 0.07
PMA, %	40.47 ± 4.56	45.56 ± 3.07	48.22 ± 2.47	38.3 ± 6.36	42.87 ± 3.55	43.3 ± 1.8
BOP, %	62.56 ± 3.38	77.02 ± 3.60	81.90 ± 7.12	62.62 ± 6.38	67.67 ± 4.07	70.3 ± 2.4

X-ray examination revealed destruction of the alveolar part of the upper and lower jaw by 1/3 of the length of the tooth root and the loss of a compact plate in the area of the tops of the interdental septa in all patients,

which indicates the generalized nature of the inflammatory-dystrophic process in the area of periodontal tissues.

Four weeks after the combination treatment with the use of an autoprobiotic or probiotic, the patients stopped complaining of bleeding, swelling, and inflammation of the gums, itching, tooth mobility, and an unpleasant odor from the oral cavity (Table 4).

TABLE 4
Changes in patients' complaints

	Group I, subgroup 1		Group I, subgroup 2		Group II, subgroup 1		Group II, subgroup 2		Control group	
	Before treatment, %	4 weeks after treatment, %	Before treatment, %	4 weeks after treatment, %	Before treatment, %	4 weeks after treatment, %	Before treatment, %	4 weeks after treatment, %	Before treatment, %	4 weeks after treatment, %
Gum bleeding during tooth brushing	100	0	100	0	100	0	100	0	100	20
Gum bleeding during eating	71.4	0	28.6	0	28.6	0	33.3	0	30	0
Spontaneous bleeding	28.6	0	14.3	0	28.6	0	33.3	0	30	0
Unpleasant odor from the oral cavity	42.8	0	42.8	0	57.1	0	66.7	0	40	0
Tooth misalignment	0	0	14.3	0	0	0	0	0	10	10
Food impaction in the interdental space	14.3	0	28.6	0	42.8	14.3	50	0	30	30
Gum itching and burning	28.6	0	28.6	0	0	0	0	0	10	0
Gum swelling and inflammation	100	0	100	0	100	0	100	0	100	10

It should be noted that after the treatment, the patients of the study and control groups showed a decrease in exudation from the periodontal pockets (Figure 1). Patients of group II, subgroup 1, and the group I, completely stopped complaining of exudation from periodontal pockets.

During treatment, patients showed a tendency towards the restoration of clinical attachment (Figure 2). There were no statistically significant differences between the results in groups I and II, as well as between subgroups ($p>0.05$).

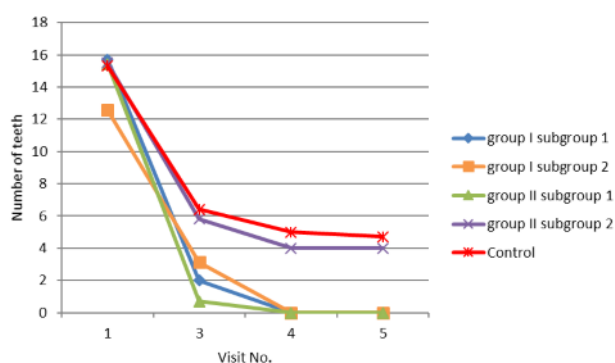


FIGURE 1.
Average number of teeth with exudation from periodontal pockets in patients of the study and control groups.

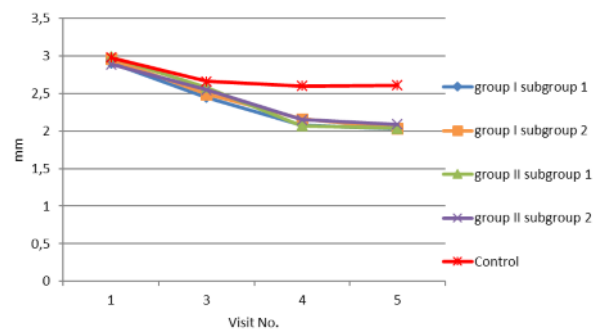


FIGURE 2

Loss of periodontal attachment in patients of the study and control groups.

Figures 3-7 show changes in oral hygiene indices in patients of the study and control groups. Already after the first application of an autoprobiotic or probiotic (groups I and II) with preliminary professional hygiene and correction of individual oral hygiene, a decrease of more than 90% in the value of the Green-Vermillion index (OHI-S) is observed (Figure 3). There was no statistically significant difference between the results of periodontitis treatment in subgroups 1 and 2 of the study and control groups ($p > 0.05$).

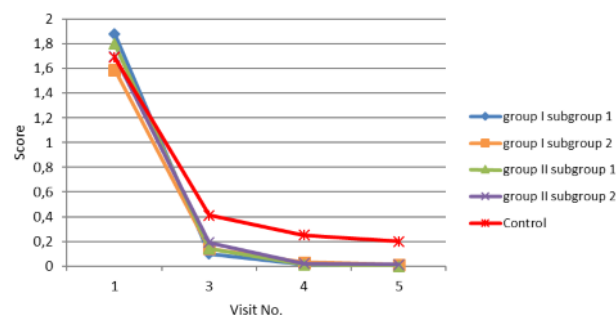


FIGURE 3.

Changes in the OHI-S index in patients of the study and control groups.

3-4 days after the start of treatment in patients of group I, the value of the PI index decreased by 93% from the initial value, and in patients of group II - by 90.9% (Figure 4). There were no statistically significant differences between the results obtained at different stages of observation of patients 1 and 2 of subgroups of the main groups and the control groups ($p > 0.05$).

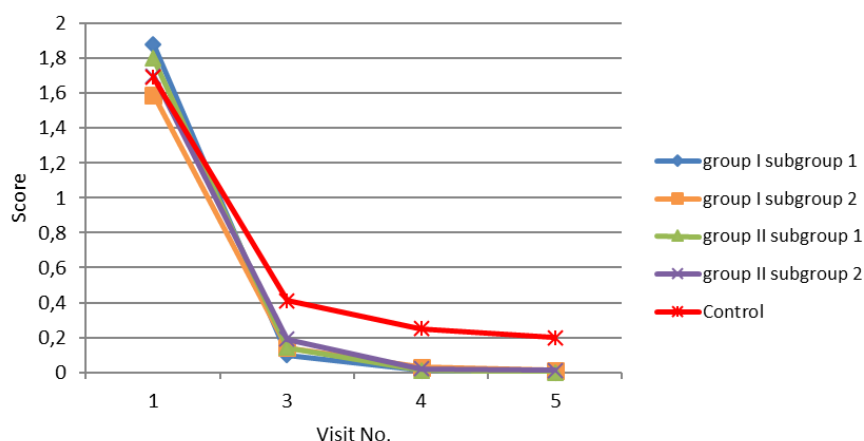


FIGURE 4

Changes in the PI index in patients of the study and control groups.

The changes in the CPITN index indicate the efficacy of combination treatment of periodontitis in patients of the control and study groups (Figure 5).

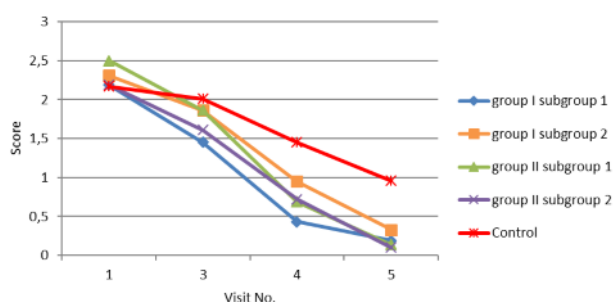


FIGURE 5.

Changes in the CPITN index in patients of the study and control groups.

3-4 days after the start of treatment, there was a decrease in inflammation in the periodontal tissues in patients of the study and control groups, as evidenced by the PMA index values (Figure 6). There were no statistically significant differences between groups I and II, as well as subgroups at all stages of patient observation ($p > 0.05$).

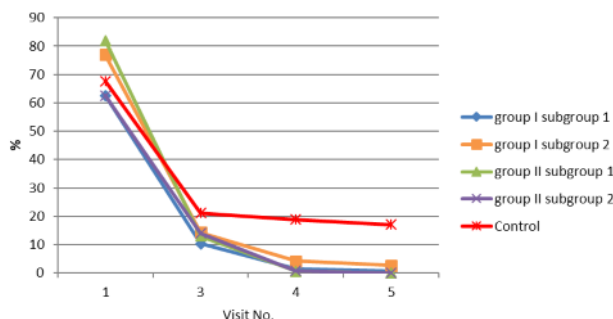


FIGURE 6

Changes in the PMA index in patients of the study and control groups.

Before treatment, all patients had a high level of bleeding of the interdental papilla and marginal gingiva (Figure 7). There were no statistically significant differences between the VOR index values in subgroups 1 and 2 of groups I and II, as well as between groups I and II ($p>0.05$).

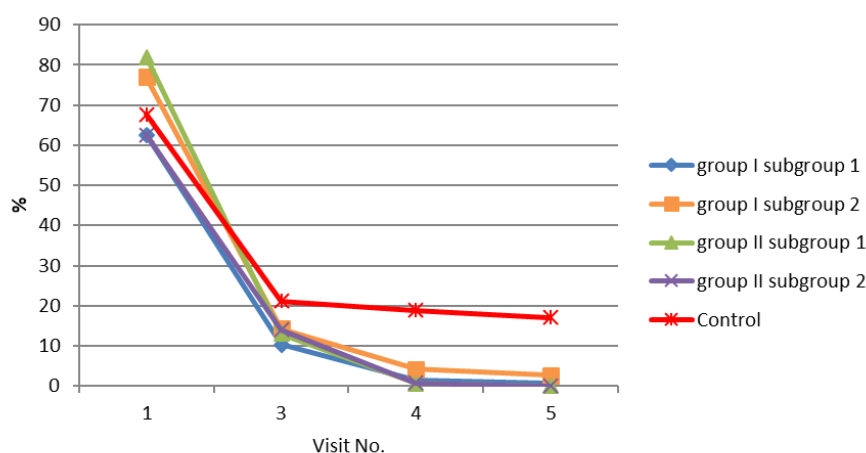


FIGURE 7.

Changes in the BOP index in patients of the study and control groups.

The efficacy of an autoprobiotic or probiotic in the combination treatment of periodontitis was monitored through the detection of an autoprobiotic or probiotic in the periodontal pockets of patients. *S. salivarius* was identified using PCR-tests. The results of detecting *S. salivarius* in cultures from periodontal pockets of patients during and after treatment are presented in Table 5. During the analysis of the data obtained, no significant differences were found in the quantitative seeding of *S. salivarius* when using an autoprobiotic and probiotic, both in the form of irrigation of periodontal pockets and in the form of oral baths ($p>0.05$). In most cases, the prevalence of *S. salivarius* was observed in the periodontal pockets of patients during and after the treatment, which suggests the contribution of an autoprobiotic or probiotic to the recovery process in the periodontal tissues.

TABLE 5

Changes in *S. salivarius* detection in the periodontal pocket cultures

	3-4 days after the first application, CFU/mL	One week after the second application, CFU/mL	4 weeks after treatment, CFU/mL
Group 1, subgroup 1	$(2.1 \pm 0.2) \cdot 10^6$	$(0.9 \pm 0.1) \cdot 10^5$	$(5.6 \pm 0.4) \cdot 10^6$
Group I, subgroup 2	$(3.7 \pm 0.2) \cdot 10^6$	$(1.4 \pm 0.2) \cdot 10^7$	$(1.5 \pm 0.2) \cdot 10^7$
Group II, subgroup 1	$(8.2 \pm 0.4) \cdot 10^6$	$(1.2 \pm 0.1) \cdot 10^7$	$(2.6 \pm 0.3) \cdot 10^7$
Group II, subgroup 2	$(6.3 \pm 0.3) \cdot 10^6$	$(2.9 \pm 0.3) \cdot 10^6$	$(5.6 \pm 0.3) \cdot 10^6$

Before the combination treatment of mild chronic generalized periodontitis, PCR-tests identified periodontopathogens of the "red complex" in all patients. The anaerobic microorganisms *P. gingivalis* dominated in the periodontal pockets (in 73% of cases). *T. forsythia* and *T. denticola* were found in 43.2% of cases and 29.7% of cases, respectively. *P. intermedia*, referring to the periodontopathogens of the "orange complex", was detected in 27% of cases.

The study of cultures from group I, subgroup 1, before combination treatment with the use of irrigation of periodontal pockets with an autoprobiotic, most often revealed the periodontopathogens of the "red

complex" *P. gingivalis* and *T. denticola*, in 57.1% of cases (Figure 8). Periodontal pathogens of the "orange complex" were not found. There was also a tendency towards the formation of microbial associations from three (14.3% of cases) periodontopathogens (*P. gingivalis*, *T. forsythia*, *T. denticola*). Combination therapy of patients with irrigation of periodontal pockets with autoprobiotics (group I, subgroup 1) led to the complete elimination of *P. gingivalis*, *T. forsythia*. At the same time, the detection rate of *T. denticola* increased from 57.1% to 85.7% of cases (Figure 8; Table 6).

The study of samples of group I of subgroup 2 before combination treatment with oral baths with autoprobiotic showed the periodontopathogen *P. gingivalis* of the "red complex" to be most often isolated - 71.4% of cases (Figure 8). The presence of representatives of only the "red complex" was established in 42.9% of cases. Also, before the start of treatment, there was a tendency to the formation of associations from two (14.3% of cases), three (28.6%), and four (14.3%) periodontopathogens studied (Table 6). Combination treatment with oral baths based on autoprobiotics (group I, subgroup 2) led to the complete elimination of *P. intermedia* and *T. forsythia*, with a relatively low level of preservation of *P. gingivalis* and *T. denticola* (28.6% and 28.6% cases, respectively). It should be noted that *P. gingivalis* was eliminated in half of the patients in this group. An improvement in the composition of the microbiota was also expressed in the disappearance of large associations from the studied periodontopathogens: only *P. gingivalis*-*T. denticola* complexes were found in the periodontal pockets of patients in 28.6% (Table 6).

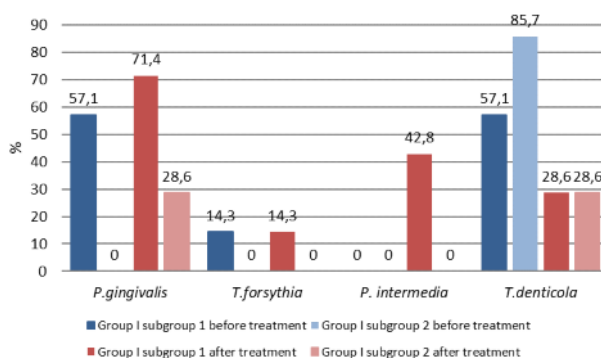
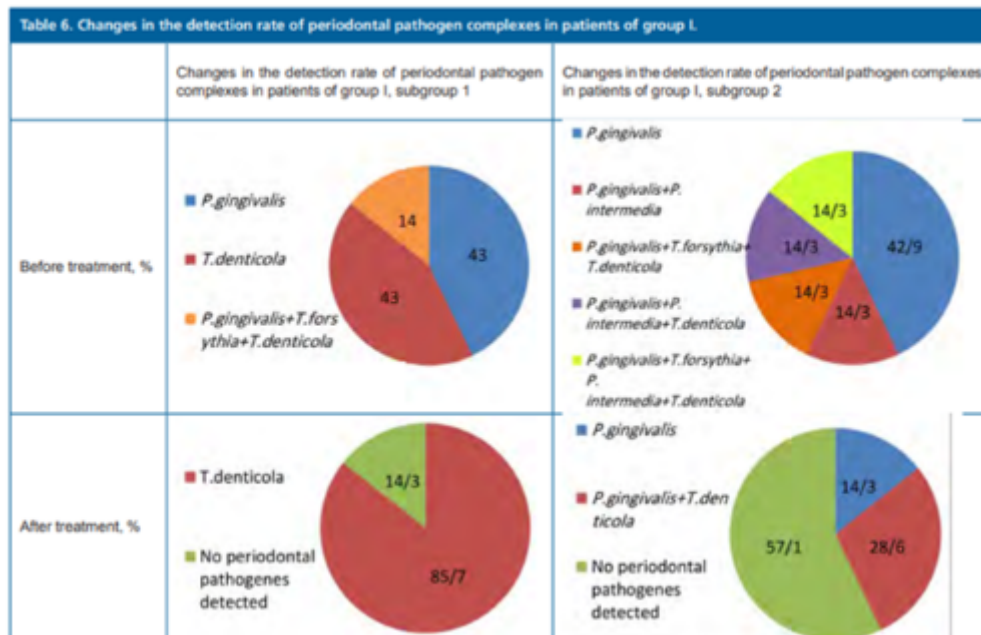


FIGURE 8.

Changes in the detection rate of periodontal pathogens in patients of group I.

TABLE 6
Changes in the detection rate of periodontal pathogen complexes in patients of group I.



Similar to the group I, the PCR-test of cultures of group II of subgroup 1 showed a predominance of the “red complex” of periodontopathogens among the patients (Figure 9). *P. gingivalis* was most often detected (71.4% of cases) among the representatives of this complex. At the same time, the formation of microbial associations from three (14.3% of cases) and four (14.3% of cases) periodontopathogens was noted (Table 7). As a result of combination treatment with irrigation of the periodontal pockets with a general probiotic (group II, subgroup 1), the previously detected periodontal pathogens were completely eliminated from the periodontal pockets of the patients (Table 7).

Before the start of the combination treatment of periodontitis with the use of a general probiotic in the form of oral baths (group II, subgroup 2), the study showed a predominance of *P. gingivalis* (83.3% of cases); in addition, *T. forsythia* (50.0% of cases), *P. intermedia* (33.3% of cases) and *T. denticola* (16.7% of cases) were also identified (Figure 9). As well as in the previous groups, the presence of microbial associations of two (33.6% of cases) and three (16.7% of cases) periodontopathogens was noted before the start of treatment ((Table 7). Combination treatment with oral baths based on a general probiotic strain resulted in the complete elimination of *T. forsythia* and *P. intermedia* and a significant decrease in the detection rate of *P. gingivalis* (up to 33.3%). The detection rate of *T. denticola* remained unchanged (Figure 9).

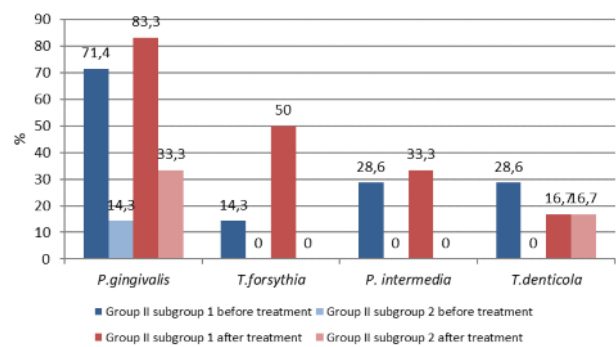
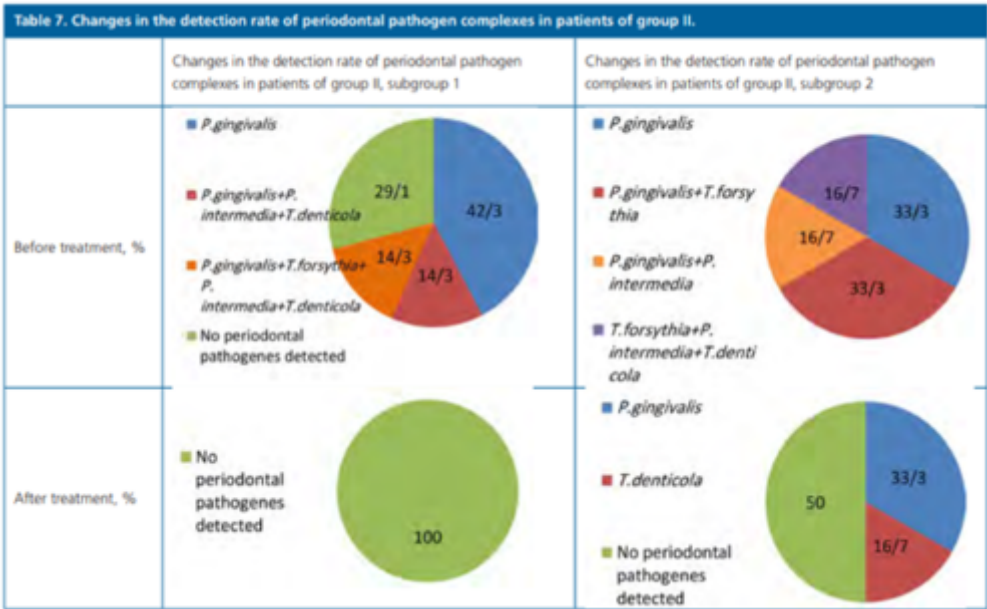


FIGURE 9.

Figure 9. Changes in the detection rate of periodontal pathogens in patients of group II.

TABLE 7

Changes in the detection rate of periodontal pathogen complexes in patients of group II



T. forsythia was dominant in the periodontal pockets of the control group of patients (100.0% of cases). *P.gingivalis* and *P. intermedia* were found in 80.0% of cases and 30.0% of cases, respectively (Figure 10). After the combination treatment of the periodontal pockets of the patients, a decrease in the previously identified periodontal pathogens was recorded simultaneously with a decrease in the number of microbial associations of two and three periodontal pathogens (20.0% of cases and 10.0% of cases, respectively). The effectiveness of the treatment, including professional hygiene and correction of individual oral hygiene, was obvious.

Nevertheless, it should be noted that the inclusion of auto- or probiotic therapy enhanced the positive effect of the performed manipulations, leading to a more pronounced effect of reducing and completely eliminating periodontal pathogens in periodontal pockets. This synergistic effect was more pronounced when periodontal pockets were irrigated with an autoprobiotic or probiotic.

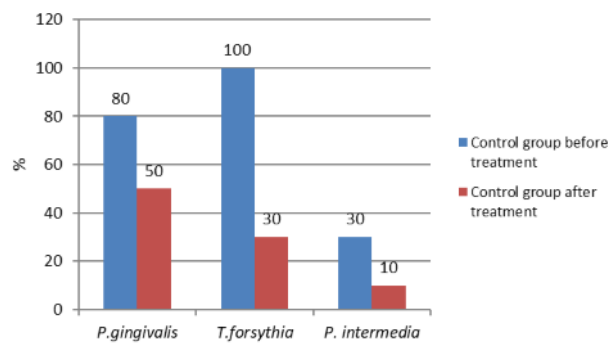


FIGURE 10

Changes in the detection rate of periodontal pathogens in patients of the control group.

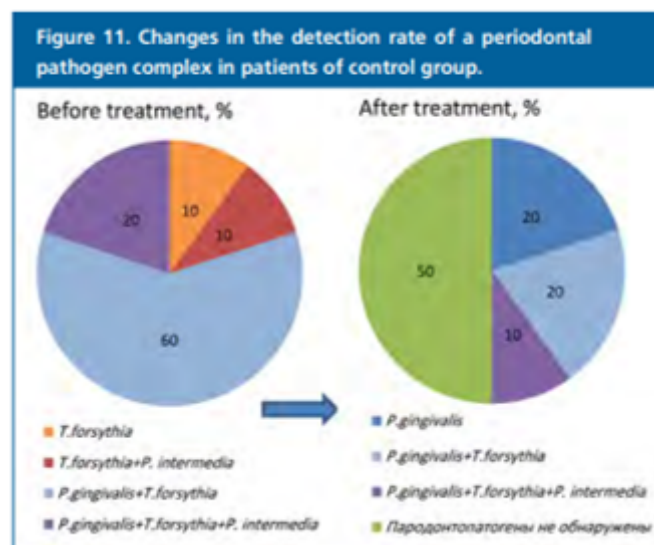


FIGURA 11.

DISCUSSION

Periodontitis is a polyetiologic disease, the leading role in the development of which is played by periodontal pathogenic microorganisms and immunological factors including mucosal immunity and the reaction of the host immune system to the specific pathogens. The impact on these factors is the basis of the conservative treatment of periodontitis¹⁻³. The efficacy of combination treatment of patients with inflammatory periodontal diseases, including local and general antibiotic therapy, is ambiguous due to the variability of periodontitis pathogens, the difficulty of predicting the course of the inflammatory process in the periodontal tissues, and the polyetiology of the disease^{5,6}. The present investigation paid special attention to the possibility of using indigenous oral bacteria autoprobiotics and probiotics based on microorganisms that are part of the normal microbiota of the oral cavity in combination with treatment of inflammatory periodontal diseases. Preference is given to bacteria with pronounced antagonistic properties aimed at inhibiting the growth of pathogenic microorganisms and restoring the normal microbiota of the oral cavity¹⁰⁻¹³. Inflammatory periodontal diseases are directly related to dysbiotic shifts in the composition of microorganisms in the oral cavity, which aggravate the severity of destructive processes in the tissues of the periodontium. Restoration of the normal microbiota of the oral cavity during combination treatment not only accelerates the recovery period but also provides reliable protection against possible relapses of the

disease. Interest in the use of autoprobiotics in the complex treatment of periodontitis is based on the concept of a personalized approach to the selection of a probiotic preparation^{16,17}. An important component of this concept is the use of microorganisms from the normal microbiota of an individual, which is a guarantee of the safety of their use for a particular person.

At the preliminary stage of this study, a group of patients with mild chronic generalized periodontitis was formed. It was found that all patients complained of bleeding while brushing their teeth, swelling, and inflammation of the gums. The hygiene indices and the state of periodontal tissues confirmed the diagnosis of chronic generalized periodontitis of mild severity. A correlation has been established between poor oral hygiene and periodontal tissue inflammation¹¹⁻¹⁷.

Four weeks after the combination treatment with the use of an autoprobiotics or probiotics, the patients stopped complaining of bleeding, swelling, and inflammation of the gums, itching, tooth mobility, and an unpleasant odor from the oral cavity. These patients showed a decrease in the values of dental indices characterizing the state of the oral cavity: a decrease in the value of the Green-Vermillion (OHI-S), PI indices to the level corresponding to good oral hygiene, as well as periodontal indices (CPITN, PMA, and BOP), which indicates the relief of inflammation in the periodontal tissues. Statistically significant differences were revealed between clinical data and index indicators of oral hygiene and the state of periodontal tissues with lower values in patients whose complex treatment included the use of an autoprobiotic or probiotic, which indicates the advisability of using an auto- and probiotic to normalize the qualitative and quantitative indicators of the state periodontal tissues²⁰⁻²³.

Thus, the use of an autoprobiotics or probiotics in the combination treatment of patients with mild chronic generalized periodontitis leads to a lasting effect: a decrease and disappearance of symptoms of inflammatory periodontal disease. Analysis of the results of clinical and index assessment of the state of periodontal tissues indicates comparable effectiveness of the use of an autoprobiotic and a probiotic in the combination treatment of mild chronic generalized periodontitis²⁴⁻²⁸.

After either autoprobiotic therapy or probiotic therapy, the dominance of *S. salivarius* was noted in the cultures from the periodontal pockets of patients, which indicates the leading role of this microorganism in the recovery processes of the microbiota of the periodontal pockets of patients².

Before treatment, all patients with mild chronic generalized periodontitis had periodontopathogens of the "red complex" *P. gingivalis* (73% of cases), *T. forsythia* (43.2% of cases), *T. denticola* (29.7% of cases), and "orange complex" *P. intermedia* (27% of cases). The data obtained are consistent with the results of previous studies²³⁻²⁵.

The combination treatment of patients with mild chronic generalized periodontitis with the use of an autoprobiotic or probiotic based on *S. salivarius* led to a more intense decrease in the detection rate of representatives of the red complex and orange complex periodontopathogens, in contrast to the control group. Evaluation of the results of a microbiological study showed comparable efficacy of using an autoprobiotic or probiotic based on *S. salivarius* in the combination treatment of chronic generalized periodontitis.

After the use of an autoprobiotic or probiotic based on *S. salivarius* in the combination treatment of chronic generalized periodontitis, there was a significant decrease in the detection rate of complexes of periodontal pathogens. Only two patients (7.4% of cases) had a complex of two periodontal pathogens (*P. gingivalis* and *T. denticola*). In contrast, patients of the control group, after treatment, had complexes of both two (20% of cases) and three (10% of cases) periodontopathogens revealed^{17,18}.

The data obtained speak for the efficacy of an autoprobiotic or probiotic based on *S. salivarius* in the combination treatment of mild chronic generalized periodontitis.

Thus, the high prevalence of periodontal diseases, the need for individual selection of drugs for effective action on periodontal pathogenic microorganisms, a small number of scientific studies on the use of

autoprobiotics in the practice of a dentist substantiates the relevance of the study to create and assess the effectiveness of the use of autoprobiotics in the treatment of inflammatory periodontal diseases^{19,20}.

As part of the combination treatment of mild chronic generalized periodontitis, local application of an autoprobiotic and/or probiotic is recommended, which improves the condition of the periodontal tissues by reducing the number of periodontal pathogens and restoring normal microbiota in the periodontal pockets of patients. Of the two proposed schemes for the use of autoprobiotic and/or probiotic, the administration of the drug in the form of irrigation of periodontal pockets seems to be the most preferred in the complex treatment of mild chronic generalized periodontitis^{7,8,12}.

CONCLUSIONS

The results of the quantitative study of the microbiota of periodontal pockets showed during the PCR screening periodontal pathogens of the "red complex" *P. gingivalis* (in 73% of cases), *T. forsythia* (in 43.2% cases), and *T. denticola* (in 29.7% of cases). *P. intermedia*, belonging to the periodontopathogens of the "orange complex" (in 27% of cases) in all patients with mild chronic generalized periodontitis. All patients showed a tendency to the formation of complexes of two or more periodontopathogens.

Analysis of the results of both clinical and index assessment of the state of periodontal tissues showed the clinical efficacy of topical application of an autoprobiotic or probiotic based on *S. salivarius* in the combination treatment of mild chronic generalized periodontitis, which consisted in normalizing the qualitative and quantitative indicators of the state of periodontal tissues. The local use of an autoprobiotic or probiotic based on *S. salivarius* in the combination treatment of inflammatory periodontal disease in patients with mild chronic generalized periodontitis causes a significant decrease in the incidence and, in some cases, the complete elimination of periodontal pathogens in periodontal pockets compared to the control group. Moreover, the irrigation of periodontal pockets with an autoprobiotic or probiotic has a more pronounced effect compared to the use of oral baths.

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