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, Saint Petersburg State University, St. Petersburg, Russia, 199034, Universitetskaya emb. 7-9, Federal State Budgetary Scientific Institution "Institute of Experimental Medicine», St. Petersburg, Russia, 197376, Academician Pavlov str., 12.
https://orcid.org/0000-0001-8952-4342
Ekaterina S. Mikhaylova, Saint Petersburg State University, St. Petersburg, Russia, 199034, Universitetskaya emb. 7-9.
https://orcid.org/0000-0003-3901-7646
Ekaterina S. Zhukovskaia, Saint Petersburg State University, St. Petersburg, Russia, 199034, Universitetskaya emb. 7-9, S.M. Kirov Military Medical Academy, St. Petersburg, Russia,194044, Academician Lebedev street lit. F. https://orcid.org/0000-0002-6928-879X
Lyudmila A. Kraeva, Saint Petersburg Pasteur Institute, St. Petersburg, Russia, 197101, Saint Petersburg, Mira str., 14,
https://orcid.org/0000-0003-1657-2479
Alexander N. Suvorov, Saint Petersburg State University, St. Petersburg, Russia, 199034, Universitetskaya emb. 7-9; Federal State Budgetary Scientific Institution "Institute of Experimental Medicine», St. Petersburg, Russia, 197376, Academician Pavlov str., 12.
https://orcid.org/0000-0002-9748-7438
*Correspondence should be addressed to Ekaterina S. Mikhaylova, Saint Petersburg State University, St. Petersburg, Russia, 199034, Universitetskaya emb. 7-9; Email: russia@prescopus.com
Received/Recibido: 12/28/2020 Accepted/Aceptado: 01/15/2021 Published/Publicado: 02/10/2021 DOI: http://doi.org/10.5281/zenodo.5102968

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 clinically and microbiologically evaluate the efficacy of autoprobiotics 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 pathogenes, 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 polietiología 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 predisposition [1]. 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 lesion[1-3].

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 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 tissues [6].

The emergence of new antibiotic-resistant strains of microorganisms, the wide spread 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[7]. 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 efficacy [8; 9; 10; 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 cytokines [12]. 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 noted [9]. Antagonism with periodontal pathogens and aggregation with oral bacteria leads to a decrease in the pathogenicity and cariogenic potential of microorganisms in the biofilm [13], as well as to a decrease in the potential load of pathogens in the oral biofilm [14]. 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 W. (2011) believes that the use of a probiotic as the only drug in the treatment of periodontitis has a weak effect [17]. Nevertheless, most studies have confirmed the efficacy of probiotics in the combination treatment of periodontitis. Penala S. (2016) considers proven the effectiveness of topical application of probiotics with L. salivarius and L. reuteri in the form of oral rinsing solutions, as three months after therapy, an improvement in the state of periodontal tissues (relief of symptoms of inflammation) was noted [11].

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 pH [18].

A modern direction in probiotic combination therapy is the assessment of the efficacy of autoprobiotics obtained on the basis of 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 histocompatibility [21]. Probiotics can have an unpredictable effect on the resident microflora: their interaction can be based on biocompatibility or on probiotic versus host and host versus probiotic antagonism [22].

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. Iliin V.K., Suvorov A.N. (2013) proved the prophylactic efficacy of autoprobiotics in the treatment of infectious and inflammatory diseases of the oral cavity and pharynx[12].

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.

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; refusal of the patient from the examination.

We used clinical, radiological and microbiological examination methods. Clinical examination of patients included taking history of life and disease, clinical assessment of the state of periodontal tissues, determination of the OHI-S hygiene index (Green, Vermillion, 1964), PI index (Silness, Loe, 1964), PMA index (Parma, 1960), BOP index (Amino, Bay, 1975) and CPITN (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 sampling of material from periodontal pockets and buccal mucosa for the cultivation of microorganisms 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 control group were carried out in 5 steps:

1) initial examination of the patient with 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

	Study group I		Study group II		Control group
	subgroup 1	subgroup 2	subgroup 1	subgroup 2	
Number of patients	**7**	**7**	**7**	**6**	**10**
Treatment	Combination treatment, including the use of an autoprobiotic based on S. salivarius in the form of irrigation of periodontal pockets (twice, with an interval of 3-4 days)	Combination treatment, including the use of an autoprobiotic based on S. salivarius in the form of oral baths (twice, with an interval of 3-4 days)	Combination treatment, including the use of a general probiotic based on S. salivarius in the form of irrigation of periodontal pockets (twice, with an interval of 3-4 days)	Combination treatment, including the use of a general probiotic based on S. salivarius in the form of oral baths (twice, with an interval of 3-4 days)	Combination treatment without the use of autoprobiotics and probiotics

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)
P. gingivalis			
Gin1	GTATATGCTCGACGAGGTGGAA	57.0	334
Gin2	ATTGTCCAGGGTAACTTCTTCG		
P. intermedia			
Int 1	AATACAGCCTTCGAGGGTTT	55.0	335
Int 2	TTCGGTCAAGACAGTAGGGA		
T. forsythia			
For1	CGAGGGTTCAATACGCTGTT	54.0	572
For2	ATAAAAATCGCATCGCAAGG		
T. denticola			
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, material was taken from the patient's cheek mucosa.

The cultivation of facultative anaerobes was carried out on 2.5% THB dense medium (Difco, USA) with the addition of 0.5% yeast extract (Helicon, Russia) and 5% ram blood at 37°C and 5% CO_2 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, an 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 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

During the study, data were obtained that were systematized in the Statistica 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 $p < 0.05$.

Results

Prior to 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±1.8% indicates the presence of moderate gingivitis in patients. Based on the high value of the BOP index (70.3±2.4%), it can be concluded that there is a high degree of bleeding in the examined patients. A correlation was found between poor oral hygiene and periodontal tissue inflammation ($r=0.74$, $p<0.05$).

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).

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 group I, completely stopped complaining of exudation from periodontal pockets.

During treatment, patients showed a tendency towards 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 ($p>0.05$). However, comparison of the results of treatment in subgroups I and II of groups with the control group revealed statistically significant differences ($p<0.01$).

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

Table 4. Changes in patients' complaints

	Group 1, 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

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

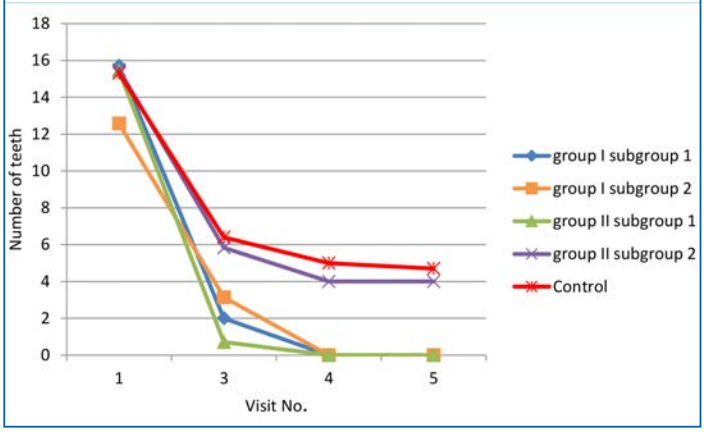
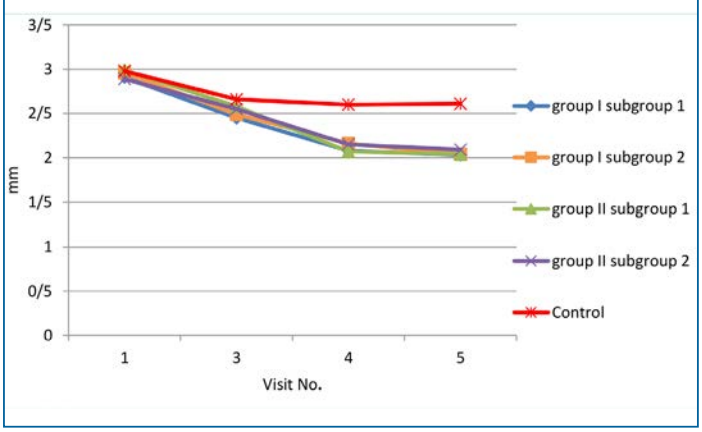


Figure 2. Loss of periodontal attachment in patients of the study and control groups.



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 ($p>0.05$). The decrease in the index during treatment in subgroups 1 and 2 of the study groups and in the control group is statistically significant relative to the initial values ($p<0.001$).

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 ($p> 0.05$). The decrease in the Silness-Loe index during the treatment of patients of subgroups 1 and 2 of the study groups and in the control group is statistically significant relative to the initial values of this indicator ($p<0.001$).

The changes in the CPITN index indicate the efficacy of combination treatment of periodontitis in patients of the control and study groups (Figure 5). Statistically significant differences were revealed between the results obtained at the final stage of observation of patients of 1 and 2 subgroups of the main groups and the control group ($p <0.05$-0.01). The decrease in the CPITN index during treatment of patients of subgroups 1 and 2 of the main groups and in the control group relative to the initial values is statistically significant ($p <0.001$ and $p <0.01$, respectively).

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 ($p>0.05$). However, when comparing the results of periodontitis treatment between the main and control groups, statistically significant differences were revealed at stages 4 and 5 of observation with the lowest PMA index values in patients of the main groups ($p<0.05$).

Before treatment, all patients had a high level of bleeding of the interdental papilla and marginal gingiva (Figure 7). After treatment, there is a decrease in the level of bleeding during probing of periodontal pockets in patients of the main and control groups relative to the initial values ($p<0.001$). 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 ($p> 0.05$). Evaluation of the BOP index at 4 and 5 stages showed significant differences between the main and control groups, which indicates the effectiveness of the use of autoprobiotics and probiotics in the course of complex treatment of periodontitis ($p<0.05$).

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.

Figure 3. Changes in the OHI-S index in patients of the study and control groups.

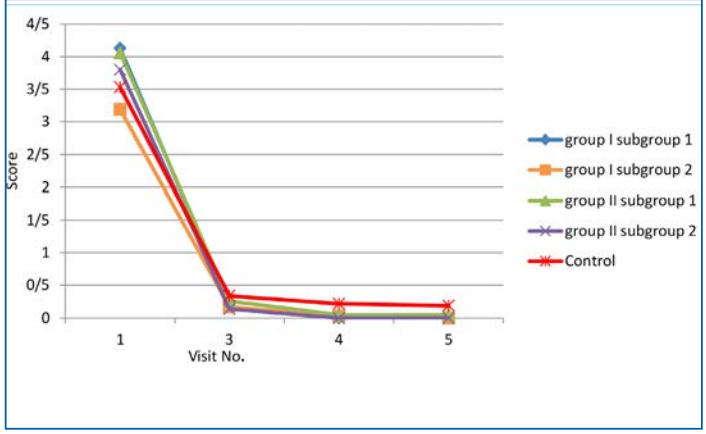


Figure 4. Changes in the PI index in patients of the study and control groups.

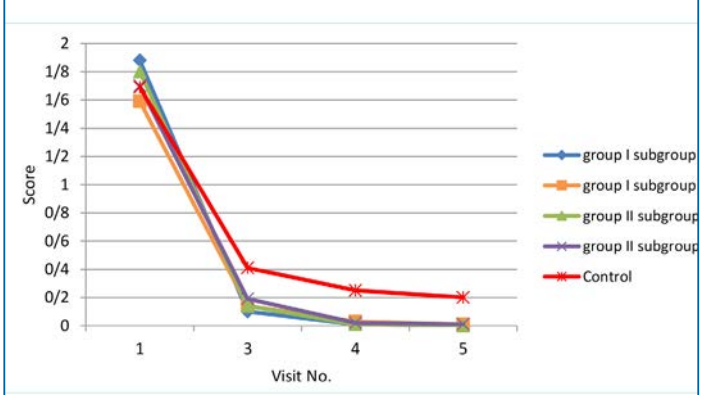


Figure 5. Changes in the CPITN index in patients of the study and control groups.

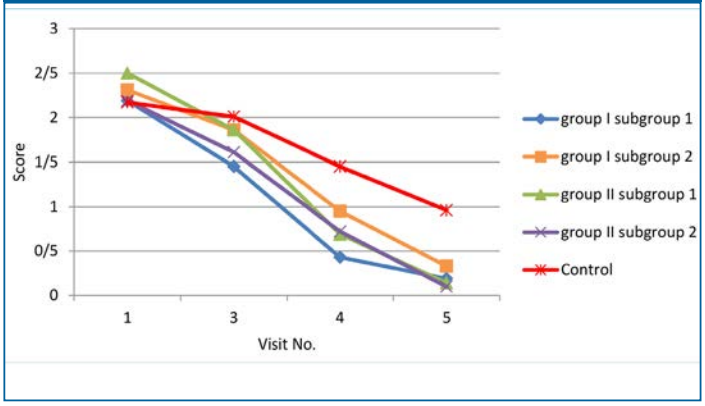


Figure 6. Changes in the PMA index in patients of the study and control groups.

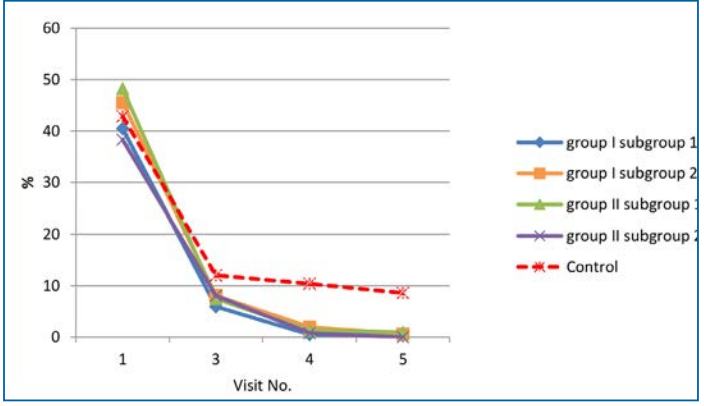


Figure 7. Changes in the BOP index in patients of the study and control groups.

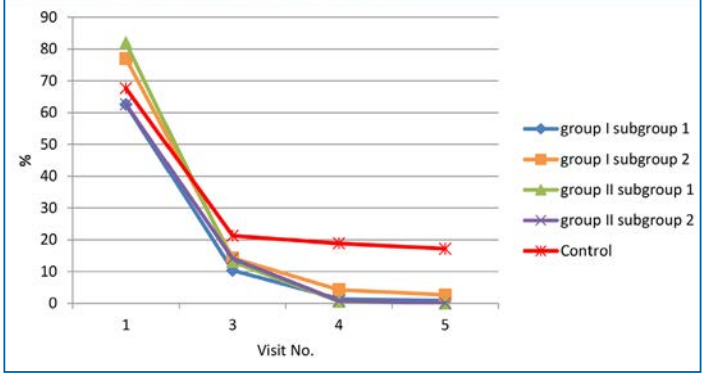


Table 5. Changes in S. salivarius detection in the periodontal pocket cultures

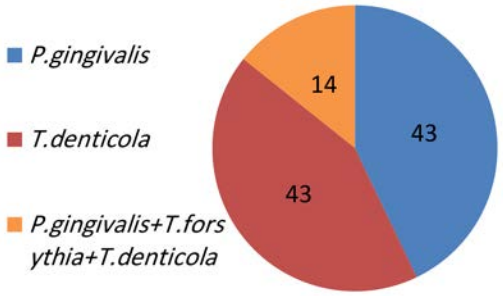
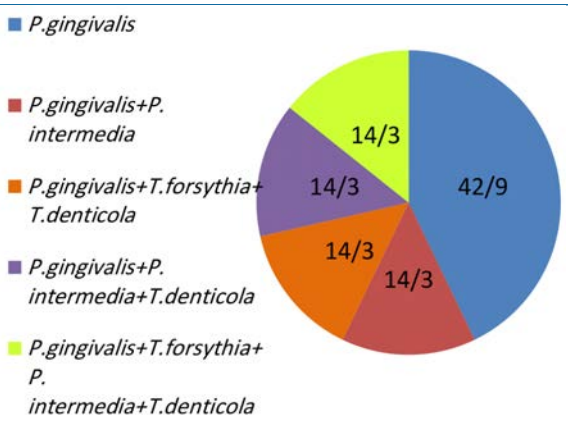
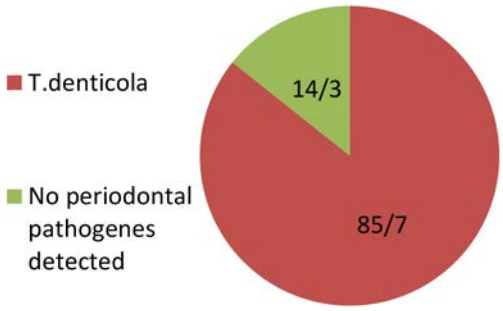
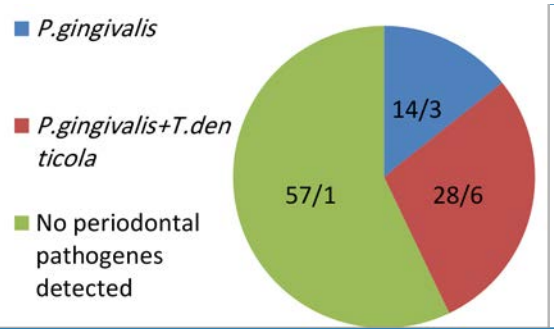
	3-4 days after the first application, CFU/ml	One week after the second application, CFU/ml	4 weeks after treatment, CFU/ml
Group 1, subgroup 1	(2.1±0.2) *10^6	(0.9±0.1)*10^5	(5.6±0.4)*10^6
Group I, subgroup 2	(3.7±0.2)*10^6	(1.4±0.2)*10^7	(1.5±0.2)*10^7
Group II, subgroup 1	(8.2±0.4)*10^6	(1.2±0.1)*10^7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

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

	Changes in the detection rate of periodontal pathogen complexes in patients of group I, subgroup 1	Changes in the detection rate of periodontal pathogen complexes in patients of group I, subgroup 2
Before treatment, %		
After treatment, %		

Similarly to group I, PCR-test of cultures of group II of subgroup 1 showed 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 in 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 8. Changes in the detection rate of periodontal pathogens in patients of group I.

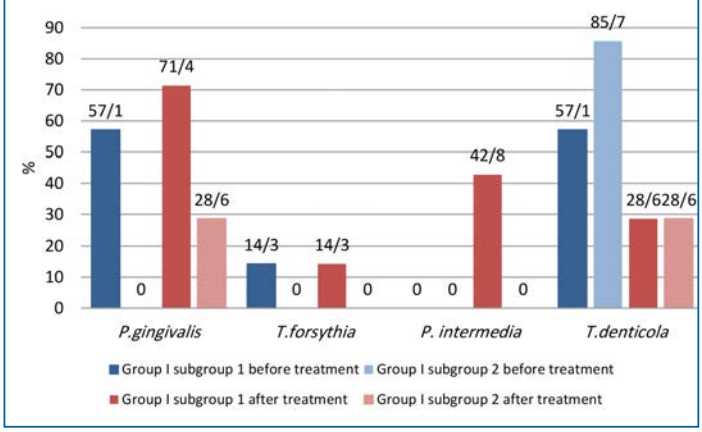


Figure 9. Changes in the detection rate of periodontal pathogens in patients of group II.

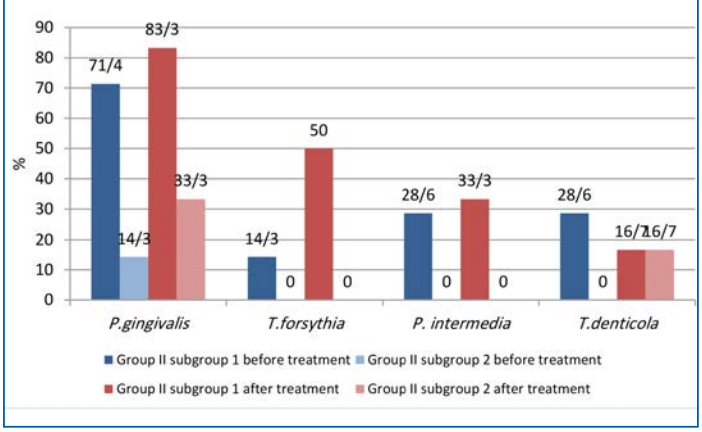
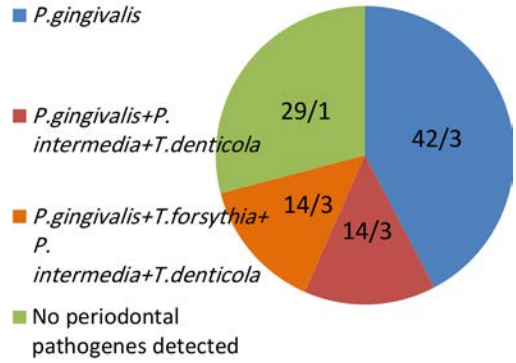
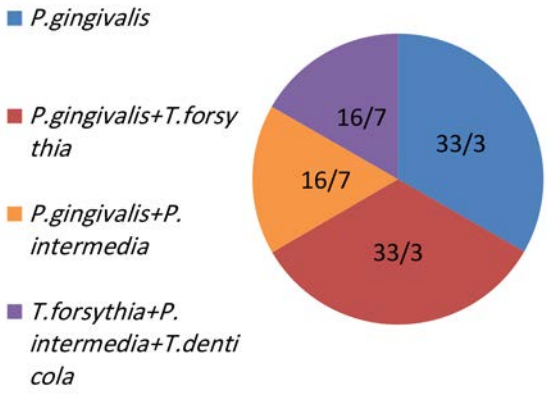
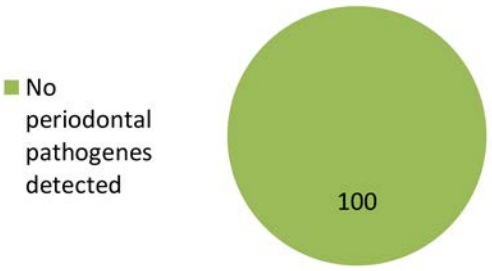
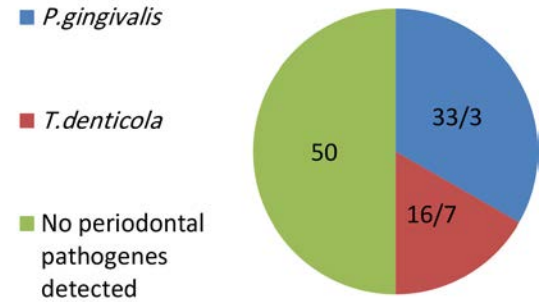


Table 7. Changes in the detection rate of periodontal pathogen complexes in patients of group II.

	Changes in the detection rate of periodontal pathogen complexes in patients of group II, subgroup 1	Changes in the detection rate of periodontal pathogen complexes in patients of group II, subgroup 2
Before treatment, %		
After treatment, %		

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). All detected microorganisms were identified in microbial associations of two and three periodontopathogens, with the exception of 10.0% of T. forsythia cases (Figure 11). 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 control group.

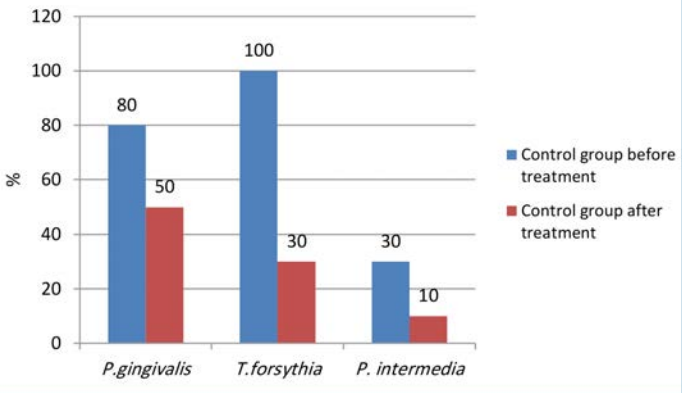
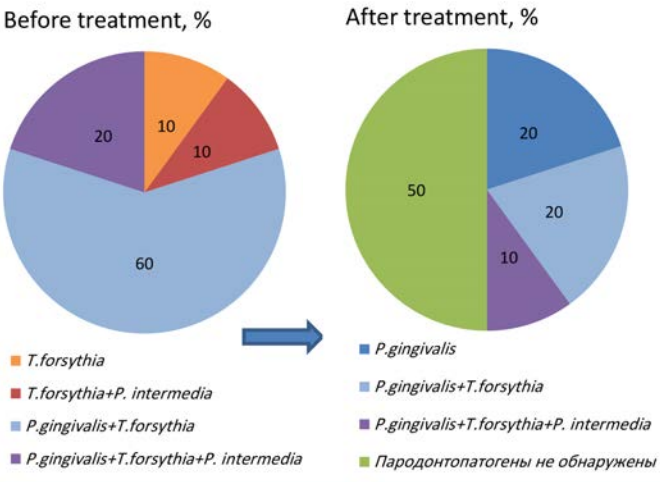


Figure 11. Changes in the detection rate of a periodontal pathogen complex in patients of control group.



Periodontitis is a polietiologic 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 periodontitis. 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. Present investigation paid special attention to the possibility of using idigenous oral bacteria autoprobiotics and probiotics based on microorganisms that are part of the normal microbiota of the oral cavity in combination 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 cavity. 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 in the course of 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. 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 indices of 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 inflammation.

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 tissues.

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 a comparable effectiveness of the use of an autoprobiotic and a probiotic in the combination treatment of mild chronic generalized periodontitis.

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 patients.

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 studies [23, 24, 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.

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 num-

ber 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.

As part of the combination treatment of mild chronic generalized periodontitis, local application of an autoprobiotic and/or probiotic is recommended, which improve 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 autobiotic 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.

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.

This study was financially supported with the Russian Science Foundation grant No. 16-15-10085 P.

References

1. Dzampaeva Z.V. Etiology and pathogenesis features of inflammatory periodontal diseases. Kuban Scientific Medical Bulletin. 2017;(5):103-110. (In Russ.) https://doi.org/10.25207/1608-6228-2017-24-5-103-110
2. Bodet C, Chandad F, Grenier D. Potentiel pathogénique de Porphyromonas gingivalis, Treponema denticola et Tannerella forsythia, le complexe bactérien rouge associé à la parodontite [Pathogenic potential of Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia, the red bacterial complex associated with periodontitis]. Pathol Biol (Paris). 2007 Apr-May;55(3-4):154-62. French. doi: 10.1016/j.patbio.2006.07.045. Epub 2006 Oct 17. PMID: 17049750.
3. Socransky S.S., Haffajee A.D. The bacterial etiology of destructive periodontal disease: Current concepts. J. Periodontol. 1992;63:322–331. doi: 10.1902/jop.1992.63.4s.322.
4. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998 Feb;25(2):134-44. doi: 10.1111/j.1600-051x.1998.tb02419.x. PMID: 9495612.
5. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135-87. doi: 10.1111/j.1600-0757.2005.00107.x. PMID: 15853940.
6. Herrera D, Alonso B, León R, Roldán S, Sanz M. Antimicrobial therapy in periodontitis: the use of systemic antimicrobials against the subgingival biofilm. J Clin Periodontol. 2008 Sep;35(8 Suppl):45-66. doi: 10.1111/j.1600-051X.2008.01260.x. PMID: 18724841.
7. Usenko D.V. Probiotics and probiotic products: possibilities and prospects of application. Current Pediatrics. 2004;3(2):50-54.
8. Seminario-Amez M, López-López J, Estrugo-Devesa A, Ayuso-Montero R, Jané-Salas E. Probiotics and oral health: A systematic review. Med Oral Patol Oral Cir Bucal. 2017 May 1;22(3):e282-e288. doi: 10.4317/medoral.21494. PMID: 28390121; PMCID: PMC5432076.
9. Mahasneh SA, Mahasneh AM. Probiotics: A Promising Role in Dental Health. Dent J (Basel). 2017 Sep 27;5(4):26. doi: 10.3390/dj5040026. PMID: 29563432; PMCID: PMC5806962.
10. Morales A, Carvajal P, Silva N, Hernandez M, Godoy C, et al. Clinical Effects of Lactobacillus rhamnosus in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up. J Periodontol. 2016 Aug;87(8):944-52. doi: 10.1902/jop.2016.150665. Epub 2016 Mar 4. PMID: 26944407.
11. Penala S, Kalakonda B, Pathakota KR, Jayakumar A, Koppolu P, et al. Efficacy of local use of probiotics as an adjunct to scaling and root planing in chronic periodontitis and halitosis: A randomized controlled trial. J Res Pharm Pract. 2016 Apr-Jun;5(2):86-93. doi: 10.4103/2279-042X.179568. PMID: 27162801; PMCID: PMC4843589.
12. Suvorov A, Karaseva A, Kotyleva M, Kondratenko Y, Lavrenova N, et al. Autoprobiotics as an Approach for Restoration of Personalised Microbiota. Front Microbiol. 2018 Sep 12;9:1869. doi: 10.3389/fmicb.2018.01869. PMID: 30258408; 4RPMCID: PMC6144954.
13. Ben Taheur F, Kouidhi B, Fdhila K, Elabed H, Ben Slama R, et al. Antibacterial and anti-biofilm activity of probiotic bacteria against oral pathogens. Microb Pathog. 2016 Aug;97:213-20. doi: 10.1016/j.micpath.2016.06.018. Epub 2016 Jun 15. PMID: 27317856.
14. Singh VP, Malhotra N, Apratim A, Verma M. Assessment and management of halitosis. Dent Update. 2015 May;42(4):346-8, 351-3. doi: 10.12968/denu.2015.42.4.346. PMID: 26062259.
15. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. J Dent. 2016 May;48:16-25. doi: 10.1016/j.jdent.2016.03.002. Epub 2016 Mar 8. PMID: 26965080.
16. Roberts FA, Darveau RP. Microbial protection and virulence in periodontal tissue as a function of polymicrobial communities: symbiosis and dysbiosis. Periodontol 2000. 2015 Oct;69(1):18-27. doi: 10.1111/prd.12087. PMID: 26252399; PMCID: PMC4530467.
17. Teughels W, Loozen G, Quirynen M. Do probiotics offer opportunities to manipulate the periodontal oral microbiota? J Clin Periodontol. 2011 Mar;38 Suppl 11:159-77. doi: 10.1111/j.1600-051X.2010.01665.x. PMID: 21323712.
18. Andreeva I.V. A new probiotic strain Streptococcus salivarius K12 in clin-

ical practice. Clinical Microbiology and Antimicrobial Chemotherapy. 2019; 21(2):92-99. doi: 10.36488/cmac.2019.2.92-99.

19. Kirilenko M.A., Kuznetsov O.Y. The creation of autoprobiotics preparation containing an active complex of bifidobacteria and lactobacilli. Mezhdunar. Nauch.-Issl. Zh. 2015; 10(41) Part 4: 61-62. doi: 10.18454/IRJ.2015.41.161.

20. Ilyin, V.K.,Kiryukhina, N.V. Disruption of the colonization resistance syndrome in humans in altered habitats and its prevention. Acta Naturae. 2014; 6(2): 11-20. doi:10.32607/20758251-2014-6-2-10-18.

21. Chicherin I.Y., Pogorelsky I.P., Lundovskikh I.A., Gavrilov K.E., et al. Autoprobiotic therapy. Journal Infectology. 2013;5(4):43-54. doi:10.22625/2072-6732-2013-5-4-43-54.

22. Glushanova NA, Shenderov BA. Relationships between the probiotic and host indigenous lactobacilli under the conditions of mixed cultivation in vitro. Zh Mikrobiol Epidemiol Immunobiol. 2005 Mar-Apr;(2):56-61. Russian. PMID: 15881941. Zorina O.A. The ratio of pathogenic representatives of the microbiocenosis of periodontal pockets in periodontitis of varying severity. Acta Naturae. 2011;3(2):103-106. doi:10.32607/20758251-2011-3-2-99-102.

23. Kulkarni MR, Bhat KG, Thomas BS, Bhat GS, Kulkarni RD. Identification of multiple strains of Porphyromonas gingivalis using heteroduplex polymerase chain reaction in varying severity of chronic periodontitis. Indian J Med Microbiol. 2018 Jan-Mar;36(1):81-86. doi: 10.4103/ijmm.IJMM_17_434. PMID: 29735832.

24. Hong BY, Furtado Araujo MV, Strausbaugh LD, Terzi E, et al. Microbiome Profiles in Periodontitis in Relation to Host and Disease Characteristics. PLoS One. 2016 Feb 3;11(2):e0148893. doi:10.1371/journal.pone.0148893.