

# Evaluation of the effect of a diode laser on the state of periodontal pathogenic microorganisms in patients with early manifestations of chronic inflammation of periodontic tissues

M.A.M. Al-Qufaish<sup>1</sup>, I.N. Usmanova<sup>2</sup>, L.P. Gerasimova<sup>2</sup>, M.M. Tuigunov<sup>2</sup>, R.F. Khusnarizanova<sup>2</sup>, A.V. Iakovleva<sup>2</sup>

<sup>1</sup>University of Aden, Yemen

<sup>2</sup>Bashkir State Medical University, Ufa, Russian Federation

## **Abstract**

**Relevance.** Chronic inflammation of periodontal tissues in young people.

**Purpose.** The aim of the work was to study of the effectiveness of the influence of a diode laser on the state of the microbiome of various biotopes of the oral cavity in patients with early manifestations of chronic inflammation.

**Materials and methods.** 70 patients (66.7%) with chronic gingivitis and periodontitis of mild severity, aged 23–35 years, were examined. A control group was consisted of 35 patients (33.3%) with clinically intact periodontium (CIP). Researchers in this group have the goal of determining critical values (22.9%) and conducting the proposed range of treatment and preventive measures. Clinical and microbiological research methods were used to assess the effectiveness of the proposed treatment and prophylactic complex.

**Results.** Our results showed high efficiency of the treatment and prophylactic complex in the treatment of early manifestations of chronic inflammation of periodontal tissues. The use of the method of simultaneous processing of the studied biotopes of the oral cavity in the complex of treatment and prophylactic measures has a positive trend. Under the influence of this effect significant elimination of periodontal pathogens of the first order and a decrease in the degree of chronic inflammation ( $p < 0.05$ ) from the initial data are observed in patients with early manifestations of chronic inflammation in periodontal tissues.

**Conclusion.** The data obtained on the basis of the study indicate that the developed treatment and oral care are an effective method for correcting the state of the microbiome of the studied biotopes.

**Key words:** clinically intact periodontium, chronic gingivitis, periodontitis, diode laser, Periopathogenic or periodontal pathogenic microflora.

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Inflammatory periodontal diseases in the modern aspect are an urgent problem, due to their high prevalence, the difficulty of diagnosing causal risk factors and, consequently, the low effectiveness of treatment and prevention measures [3, 5, 9, 11, 12, 14, 15, 18, 19].

According to the WHO data (2016) and data from the Russian Society of Periodontology and the European Federation of Periodontology in Russia and the world as a whole, signs of early inflammatory processes and chronic inflammation are detected in more than 95% of young people, while 16% of those who applied for a preventive examination have already taken place clinical signs of chronic inflammation with the presence of dental or periodontal pockets. In this regard, the problem of improving early diagnosis, conducting high-quality medical and preventive measures does not lose its relevance [1-3, 4, 6, 7, 12-19, 23].

Initial manifestations of inflammatory processes in the periodontium are caused by high reserve, immune and reparative features, periodontal tissues may correspond to the norm, or proceed without a pronounced clinical picture and subjective complaints of the patient [7, 20, 22, 24, 26].

The chronization of the inflammation process is not always accompanied by pronounced clinical manifestations, but the complaints of patients can be quite justified. Lesions of periodontal tissues in the presence or absence of clinically chronically expressed inflammation are often associated with the degree of resorption, the depth of the

dental or periodontal pocket, changes in the microbiome of the oral cavity, and consequently pathological reactions occurring in the tissues [3, 8, 11, 21, 25, 27].

Thus, the implementation of modern therapeutic and preventive measures for early manifestations of chronic inflammation in periodontal tissues with the ability to fully reduce the microbial potential is relevant and promising, which caused the relevance and purpose of our study.

## **PURPOSE**

To evaluate the effectiveness of local treatment of early manifestations of chronic periodontal tissue inflammation in young people using a diode laser.

## **MATERIAL AND METHODS**

Our comprehensive survey program was conducted in 2 stages. The first stage of the study included a comprehensive clinical dental examination with an assessment of dental status data, assessment of the clinical condition of periodontal tissues, and determination of hygienic and periodontal indices. The second stage of PCR diagnostics allowed us to conduct a qualitative and quantitative analysis of the composition of the microbiome of the studied oral biotopes, and the ability to identify and determine the critical titers of periodontal pathogens of diagnostic significance. In individuals with clinically intact periodontal disease, this method allowed us to determine the risk of developing inflammatory diseases, in individuals with ear-

ly manifestations of chronic inflammation in periodontal tissues – to predict the dynamics and stabilization of the process, to justify the recommended algorithm of treatment and prevention measures with an assessment of their effectiveness.

We conducted a comprehensive clinical dental examination of 105 young people, who were divided into two main clinical groups and a comparison group.

The comparison group consisted of 35 patients (33.3%) with clinically intact periodontal disease (IP). The study was conducted to clarify the titer values of the studied periodontal pathogens, as well as an index assessment of the condition of periodontal tissues. In 22.9% of cases of PCR studies, the risks of developing chronic inflammation were identified in the form of high titers of periodontal pathogenic microflora, which was corrected using the proposed complex of therapeutic and preventive measures. The remaining 77.1 per cent of individuals with identified low thresholds titles parodontopathogenic microflora also conducted treatment and preventive measures according to the standard scheme is STAR, 2001.

35 patients (33.3%) of the first clinical group with diagnosed chronic gingivitis (CG) and critical titles of periodontopathogenic microorganisms was subjected to complex treatment and preventive measures, which allowed at 42.9% of the cases to stabilize the inflammatory process in periodontal tissues.

In the second clinical group consisting of 35 people (33.3%) with chronic generalized periodontitis of mild severity and identified high titers of periodontopathogenic microorganisms, the proposed complex was performed in 65.7% of cases, and in 34.3% of cases with identified low threshold values of titers, therapeutic and preventive measures were performed according to the generally accepted scheme STAR, 2001.

All clinical groups are comparable in age and gender.

Clinical assessment of tissue included determination of the index bleeding SBI simplified hygiene index OHI-S popularno-marginal-alveolar index PMA, gingival index, PI, analysis of data obtained in a clinical dental examination, and analysis of data obtained by x-ray (radiography dental computer) and microbiological examination methods. All the parameters under study are based on the definition standards of who, the Russian Society of Periodontology and the European Federation of periodontics (EFP).

The criteria for inclusion in the studied clinical groups were the presence of informed consent of patients, male and female persons aged 20 to 35 years, without the presence of somatic diseases, and the absence of high-quality therapeutic and preventive measures in the oral cavity for the last 6 months. Criteria for exclusion to the study clinical groups: lack of informed consent of patients, men and women over 35 years of age, persons with the presence of somatic diseases and with high-

quality medical and preventive measures carried out at the time of examination.

To study the spectrum and number of studied periodontopathogenic microorganisms of both the first and second order, all patients took biological material from the studied oral cavity biotopes – dental plaque, gingival and oral fluid, and the contents of the periodontal pocket (table 1).

Identification of isolated cultures was carried out based on the study of their biochemical properties using special test systems.

The process of DNA isolation takes about 20 minutes on average and consists of alternating three stages: moving a test tube with a DNA EXPRESS reagent containing the analyzed material for 10 seconds, warming the test tube in a solid-state thermostat at 98°C for 20 minutes and separating the supernatant containing DNA by centrifugation at 8,000-14,000 rpm for 20-30 seconds.

Microbiological experiments of the material were performed in dynamics before the complex of therapeutic measures and after 14 days, 3 months, 6 months, and 12 months.

Amplification of species – specific DNA fragments of the studied bacteria-porphyrromonas of blood, Treponema denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, P. endodontalis, Fusobacterium nucleatum, Prevotellum promezh was performed using the polymerase chain reaction (PCR) method using specific primers in the multichannel amplifier Terzik MS-2 (NPF "DNA technology", Russia).

To study the spectrum and number of studied periodontopathogenic microorganisms of both the first and second order, all patients took biological material from the studied oral cavity biotopes – dental plaque, gingival and oral fluid, and the contents of the dental pocket. Using the method of PCR diagnostics (test system "Dentoscreen "NPF" Litech") allowed to identify – Porphyromonas gingivalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis, P. endodontalis, Fusobacterium nucleatum, Prevotella intermedia.

Local treatment of early manifestations of chronic inflammation in periodontal tissues due to the presence of high threshold values of periodontal pathogenic microflora titers was performed using PICSSO Lite diode laser in combination with laminaria containing gel in the form of gel and adhesive plates.

There were no contraindications for diagnostic and therapeutic measures in these patients.

All the obtained data were subjected to mathematical calculations – arithmetic mean series, their errors, standard deviations were calculated, and the student's confidence coefficient was determined Excel, Excel-2000. Quantitative characteristics were described using the arithmetic mean (M) and standard error of the mean (m). When describing qualitative characteristics, relative

Table 1. Collection, delivery and storage of biological material

Biotope	Taking a sample for analysis
Plaque	Taking dental plaque with a sterile universal probe of type A. place the Tip of the probe with plaque in a test tube with "DNA EXPRESS", make 5-10 rotational movements with the probe, then remove the probe and close the lid
Gingival liquid	Select with sterile paper strips of 0.3-0.8 mm or paper pins (endodontic paper pins) №20-40 for 10-20 seconds. Put in a test tube with "DNA EXPRESS"
Contents of the periodontal pocket	Sterile paper pins (endodontic paper pins) №20-40. Place in the periodontal pocket for 10-20 seconds. Remove the pins and place them in a test tube with "DNA EXPRESS"

shares and standard error of the share were calculated, and the reliability of the results was evaluated using the reliability criterion – T (student's criterion). Statistical hypotheses were tested by comparing the obtained significance level (p) with the threshold level of 0.001; 0.01; 0.05. At  $p \leq 0.05$ , the null hypothesis that there were no differences between the indicators was rejected and an alternative hypothesis was accepted. Statistically significant values were considered ( $p < 0.05$ ).

**RESULTS AND DISCUSSION**

A comprehensive dental examination was performed in 105 young people without severe somatic pathology. The patients under observation, depending on the clinical condition of periodontal tissues, were distributed as follows: 35 (33.3%) patients showed slight pronounced inflammation of periodontal tissues in intact periodontal disease (IP), and 8 (7.62%) of them had risk factors in the form of the presence of periodontal pathogenic microflora, 35 (33.3%) had chronic gingivitis (By 05.1), and 35 (33.3%) patients had chronic periodontitis (by 05.31). Patients with intact periodontal disease in 7.62% of cases complained of itching and spontaneous bleeding when brushing their teeth and eating hard food. All patients with chronic gingivitis and periodontitis of mild severity most often complained of bleeding when brushing their teeth and eating hard food, periodically appearing aching pain in the gums and discomfort in the gums. Upon objective examination, these individuals have gingival papillae that are swollen, enlarged, loose, hyperemic, with a bluish tinge; the gingival margin is edematous, enlarged in volume, roller-like thickened, the marginal part of the gum is edematous, cyanotic.

The hygienic state of the oral cavity on average turned out to be poor in all clinical groups, as evidenced by the values of the green-Vermillion index-more than 2 points (with a norm of 0.0-0.6), the prevalence of symptoms such as bleeding gums and the presence of hard dental deposits, according to who criteria, was low and average, respectively, of the norm, as a result of which the indices of PMA, GI and SBI ( $p \leq 0.05$ ) (Fig. 1).

The student's test, with the null hypothesis that there were no differences in the mean values in the two samples, showed at a significance level of  $p < 0.001$  that all samples of CG and CGPLS differed statistically signifi-

cantly from the comparison group for all the considered indices, and the groups also differed statistically significantly ( $p < 0.001$ ).

The analysis of 3D CT in 7.62%±1.75% of individuals with intact periodontal disease revealed changes in the external and internal cortical plate in the form of its thinning in the area of individual teeth, small foci of osteoporosis at the tops of the alveolar ridges, which corresponds to the initial radiological signs of chronic inflammation. Densitometric studies in these individuals showed that in the area of the middle of the vertexes of the interdental partitions in the Central teeth of the lower jaw, the average bone density was  $1398.00 \pm 53.42$  y. e., in the area of the chewing group of teeth of the lower jaw –  $1567.00 \pm 49.64$  y. e., in the upper jaw in the area of the Central teeth  $1166.00 \pm 46.58$  y. e., in the area of the chewing group of teeth  $1585.00 \pm 51.31$  y.e.

The study subjects with chronic gingivitis and periodontitis of mild severity revealed the expansion of the periodontal gap and thinning of the dental partitions in the area of individual teeth. Densitometry in the middle of the vertexes of the interdental septum in the Central teeth of the lower jaw averaged  $1458.00 \pm 46.35$  y.e., in the area of the chewing group of the lower jaw –  $1597.00 \pm 51.22$  y.e., in the upper jaw in the area of the Central teeth  $1256.00 \pm 33.54$  y.e., in the area of the chewing group of teeth  $1599.00 \pm 47.34$  y.e.

Microbiological research of various oral cavity biotopes (dental plaque, gingival and oral fluid, periodontal pocket contents) in all groups, regardless of the clinical state of periodontal tissues, allowed to identify periodontal pathogenic microorganisms of various complexes.

Among the representatives of the red complex, the most common subspecies were *Treponema denticola* from 20.0% to 28.6% of cases, *Tannerella forsythia* from 11.4% to 20.0% and *P. gingivalis* from 22.9% to 71.4% of cases (Fig. 2).

The orange complex was a representative of aerobic gram-negative microorganisms-*Fusobacterium nucleatum* detected on average from 34.3% to 54.3% of research cases. Among the representatives of the green complex in the studied biotopes, *Aggregatibacter actinomycetemcomitans* serotype a was most often detected (Fig. 3).

In patients with clinically intact periodontal disease (TRC) in the studied biotopes, *Fusobacterium*

**Table 2. The proportion of positive samples for the identification of conditionally pathogenic and obligate-anaerobic microorganisms in the biotopes of the oral cavity in young people**

Types of microorganisms	IP (n = 35) plaque, gingival fluid		Chronic gingivitis (n = 35) gingival fluid, oral fluid		Chronic periodontitis (n = 35) periodontal pocket contents, oral fluid	
	abc.	%	abc.	%	abc.	%
Laktobatsilly	17	48,57	31	88,57*	23	65,71**
Candida spp. (C. albicans)	8	22,85	23	65,71*	22	62,86**
p. Neisseria	29	83%	19	54,7%	20	57,4%
Str. mitis, Str. sangius	35	100%	29	82,85%	32	91,4%
Treponema denticola	7	20	8	22,86%	10	28,57**
Porphyromonas gingivalis	8	22,85	17	48,57*	25	71,42**
Porphyromonas endodontalis	4	11,43	8	22,86*	13	37,14**
Aggregatibacter actinomycetemcomitans	5	14,29	13	37,14*	16	45,71**
Fusobacterium nucleatum	12	34,28	17	48,57*	19	54,28**
Tannerella forsythia	4	11,43	6	17,14*	7	20**

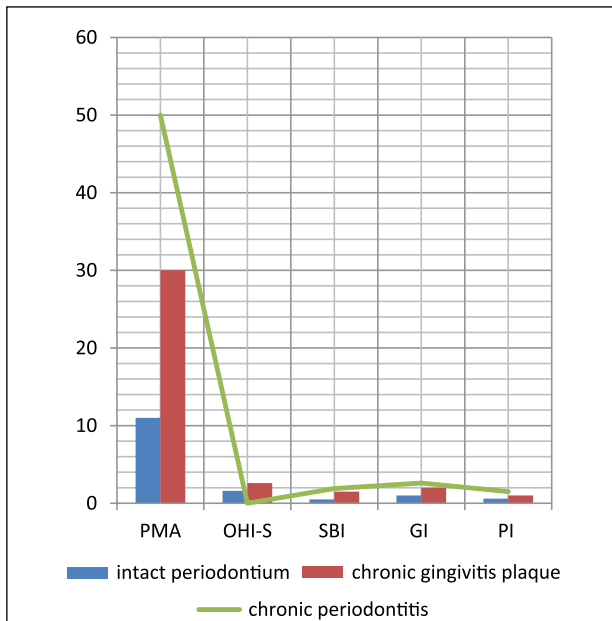


Fig. 1. Values of index assessment of periodontal tissue state depending on the clinical condition

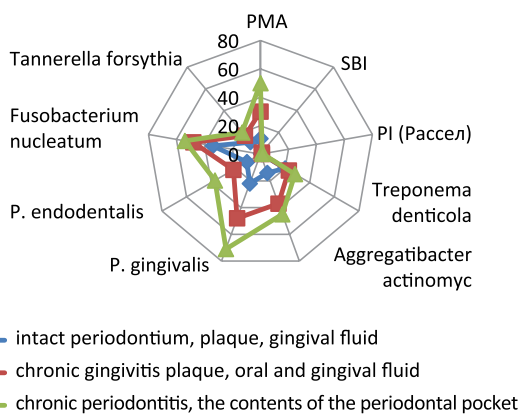


Fig. 4. The qualitative ratio of the identified representatives of periodontopathogenic microflora depending on the clinical condition of periodontal tissues and on their degree of pathogenicity

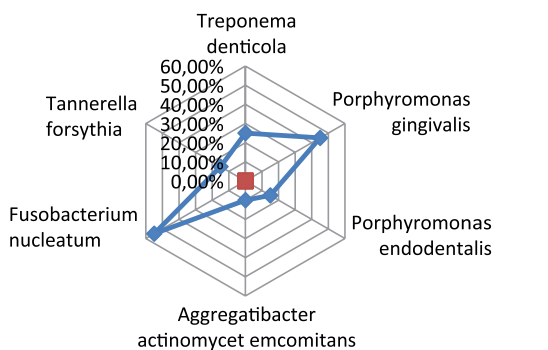


Fig. 6. Dynamics of the influence of the therapeutic and prophylactic complex on changes in the titers of periodontal pathogenic microflora in the group with chronic gingivitis

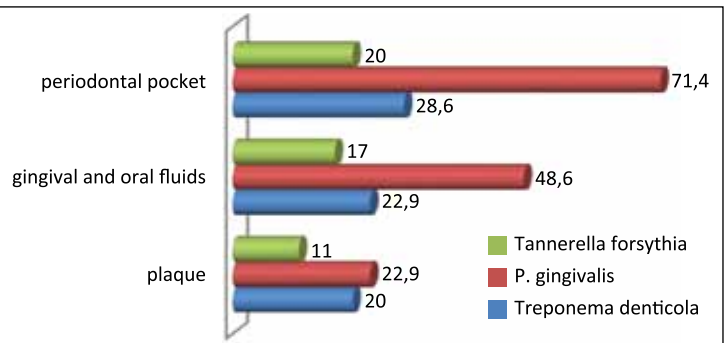


Fig. 2. Quantitative ratio of periodontopathogenic microorganisms belonging to the red complex in the studied biotopes in percent

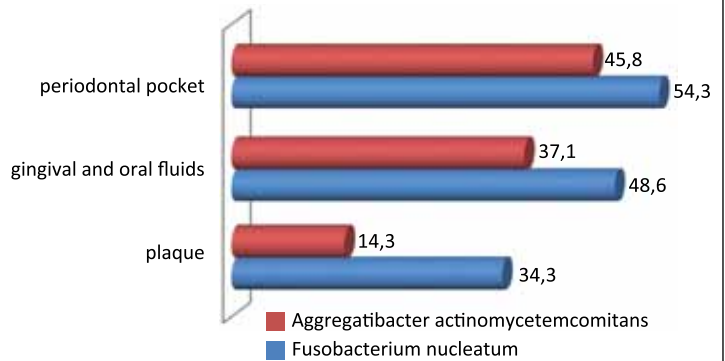


Fig. 3. Quantitative ratio of periodontopathogenic microorganisms belonging to the orange and green complex in the studied biotopes in percent

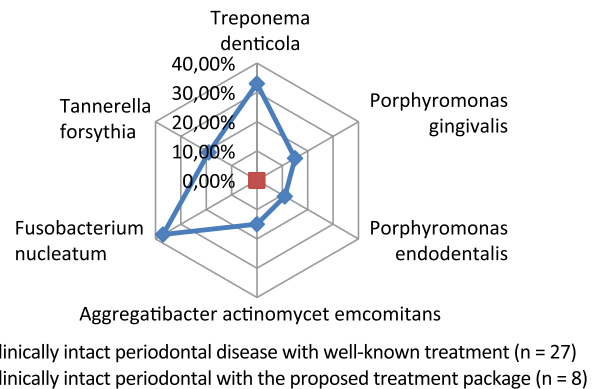


Fig. 5. Dynamics of the influence of the therapeutic and prophylactic complex on changes in the titers of periodontal pathogenic microflora in the comparison group

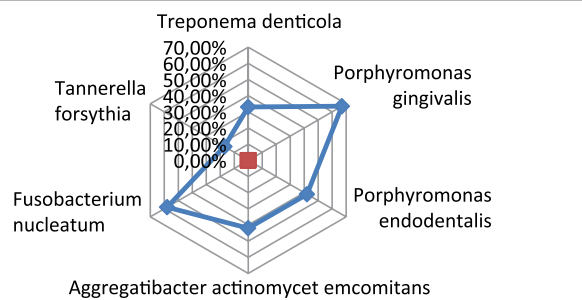


Fig. 7. Dynamics of the influence of the therapeutic and prophylactic complex on changes in the titers of periodontal pathogenic microflora in the group with mild chronic periodontitis

nucleatum was detected in 34.28%, Porphyromonas gingivalis – 22.85%, Aggregatibacter actinomycetemcomitans – 14.29% of cases. When diagnosed with chronic gingivitis (CG) and periodontitis (CP), the frequency of their occurrence is significantly higher, respectively, for Porphyromonas gingivalis by 2 times, for Fusobacterium nucleatum by 1.5 times, for Aggregatibacter actinomycetemcomitans by 2.6 and 3.2 times (table 2).

In most cases, the analysis of data obtained during microbiological studies by PCR in patients with clinically intact periodontal disease, chronic gingivitis and periodontitis of mild severity in the studied biotopes of the oral cavity revealed periodontopathogenic microorganisms in critical or low titers (Fig. 4).

Detection of the presence of representatives of the orange and red complex, regardless of the clinical state of periodontal tissues, is closely related to the identified clinical indicators, in particular, with bleeding gums during probing, the presence of chronic inflammation and periodontal pocket.

Analysis of data obtained before the proposed treatment package showed that high DNA titers of the desired periodontal pathogens were identified in 46 patients (43.8%), including 8 patients with clinically intact periodontal disease (22.85%), with chronic gingivitis in 15 patients (42.85%) and 23 patients with mild chronic periodontitis (65.71%). The treatment contributed to their significant reduction ( $\chi^2 = 8.57$ ,  $p = 0.003$ ). The best results were observed in the dynamics of reducing the content of first-order periodontal pathogens *T. denticola*, *P. gingivalis* and *A. actinomycetemcomitans* (Fig. 5-7).

In patients with clinically intact periodontal disease with high titers, the proposed treatment complex resulted in complete elimination of plaque and gingival fluid in the biotope of *P. gingivalis*, *A. Actinomycetemcomitans*, *T. denticola*, and *P. endodontalis*, with a 3- and 2-fold decrease in *Fusobacterium nucleatum* and *Tannerella forsythia*, respectively ( $p \leq 0.05$ ) (Fig. 5).

In patients with chronic gingivitis, the treatment complex also contributed to the qualitative elimination of periodontopathogenic species belonging to the first order, while the content of *P. endodontalis*, *Fusobacterium nucleatum* and *T. forsythia* decreased quantitatively 3.4, 3.7 and 2.6 times with a statistically significant difference than in the first study (Fig. 6).

When diagnosed with chronic generalized periodontitis mild the complex of local treatment also contributed to their elimination and reduction in quality *P. endodontalis*, *Fusobacterium nucleatum* and *T. forsythia*, respectively, 2, 4 and 1.5 times ( $p \leq 0.05$ ) (Fig. 7).

The best effect was achieved in patients with the proposed method of local treatment in respect of complete elimination of *T. denticola*, *P. gingivalis*, *A. actinomycetemcomitans*, *P. endodontalis* и снижении *Fusobacterium nucleatum* и *Tannerella forsythia* ( $\chi^2 = 0,99$ ,  $p = 0,0009$ ).

Depending on the clinical state of periodontal tissues, low titers of the desired types of periodontal pathogens were detected in 59 patients (56.1%), including 27 patients with TRC (77.14%), 15 patients with CG (57.14%) and 23 patients with CGPLST (65.71%). The complex of well-known treatment contributed to a decrease ( $\chi^2 = 0.99$ ,  $p = 0.0009$ ), however, their complete elimination was not observed.

The studied periodontopathogenic microorganisms were detected before and after local treatment – *T. denticola* was detected in 18 patients, *P. gingivalis* in 21, and *A. actinomycetemcomitans* in 11 patients regardless of the clinical condition of periodontal tissues ( $\chi^2 = 13.47$ ,  $p = 0.0002$ ;  $\chi^2 = 9.26$ ,  $p = 0.002$ ;  $\chi^2 = 4.00$ ,  $p = 0.046$ ).

## CONCLUSION

1. The use of a therapeutic and prophylactic complex provides a significant increase in the therapeutic effect and an increase in the remission period for early manifestations of chronic inflammation in periodontal tissues.

2. The effect of a diode laser has a pronounced anti-microbial effect against periodontal pathogenic microorganisms located in various biotopes of the oral cavity and inaccessible to mechanical and antiseptic agents.

3. The results obtained by us showed a high effectiveness of the use of a therapeutic and prophylactic complex in the complex of local treatment of early manifestations of chronic inflammation in periodontal tissues in young people with identified risk factors in the form of high titers of periodontal pathogenic microflora.

Thus, the proposed complex of local treatment of early manifestations of chronic inflammation in periodontal tissues contributes to the complete elimination of periodontal pathogenic microflora of the first order.

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## СВЕДЕНИЯ ОБ АВТОРАХ / INFORMATION ABOUT THE AUTHORS

**Мохаммед Али Мохаммед Аль Кофиш**, к.м.н., врач-стоматолог Стоматологического факультета Университета г. Аден, республика Йемен

alqufaesh@gmail.com

ORCID: <https://orcid.org/0000-0002-3065-6831>

**M.A.M. AL-QUFAISH**, Associate Professor, dentist Faculty of Dentistry, University of Aden, Yemen

**Усманова Ирина Николаевна**, д.м.н., профессор кафедры терапевтической стоматологии с курсом Института дополнительного профессионального образования Федерального государственного бюджетного образовательного учреждения высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Уфа, Российская Федерация

irinausma@mail.ru

ORCID: <https://orcid.org/0000-0002-1781-0291>

**Usmanova Irina N.**, DSc, Professor of the Department of therapeutic dentistry with the course of Institute of additional professional education of the Federal State Bashkir Medical University of the Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation

**Герасимова Лариса Павловна**, д.м.н., профессор, заслуженный врач РБ, заведующая кафедрой терапевтической стоматологии с курсом Института дополнительного профессионального образования Федерального государственного бюджетного образовательного учреждения высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Уфа, Российская Федерация

gerasimovalarisa@rambler.ru

ORCID: <https://orcid.org/0000-0002-1145-6500>

**Gerasimova Larisa P.**, DSc, Professor, Honored Doctor of the chief of the Department therapeutic dentistry with the course of Institute of additional professional education of the Federal State Bashkir Medical University of the Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation

**Туйгунов Марсель Маратович**, д.м.н., профессор, заведующий кафедрой микробиологии и вирусологии Федерального государственного бюджетного образовательного учреждения высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Уфа, Российская Федерация

tuynunov@mail.ru

ORCID: <https://orcid.org/0000-0002-5473-2034>

**Tuynunov Marcel M.**, DSc, Professor, head of the Department of Microbiology and Virology of the Federal State Bashkir Medical University of the Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation

tuynunov@mail.ru

ORCID: <https://orcid.org/0000-0002-5473-2034>

**Хуснарязанова Рауза Фазыловна**, к.м.н., доцент кафедры микробиологии и вирусологии Федерального государственного бюджетного образовательного учреждения высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Уфа, Российская Федерация

roza.khusna@mail.ru

ORCID: <https://orcid.org/0000-0002-1001-9587>

**Khusnarizanova Rausa F.**, PhD, Associate Professor of the Department of Microbiology and Virology of the Federal State Bashkir Medical University of the Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation

**Яковлева Анастасия Владимировна**, студентка 5 курса стоматологического факультета Федерального государственного бюджетного образовательного учреждения высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации, г. Уфа, Российская Федерация

Anastasiadantist21@yandex.ru

ORCID: <https://orcid.org/0000-0003-1123-4438>

**Iakovleva Anastasia V.**, 5th year student of the faculty of dentistry of the Federal State Bashkir Medical University of the Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation



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