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Peculiarities of oral microbiota in patients with small and medium-sized dental defects and chronic periodontitis of moderate severity

Oksana A. Shuliatnikova, Mikhail V. Yakovlev, Anatoliy P. Godovalov

Perm State Medical University named after Academician E.A. Wagner, Perm, Russia

ABSTRACT

BACKGROUND: Chronic generalized periodontitis is one of the most common dental pathologies and causes difficulties in adopting rational treatment plan, since there are no biomarkers to qualitatively assess the possibility of developing periodontal inflammation or its pathomorphosis.

AIM: Study of peculiarities of oral microbiota in patients with acquired dentition defects under chronic generalised periodontitis of moderate severity.

MATERIALS AND METHODS: The study participants were 88 people (44 males, 44 females) with small/medium dental defects (10th International Classification of Diseases — K08.1), who were divided into two groups: the first — without clinical signs of periodontal inflammation (23 men, 21 women); the second — with chronic generalized periodontitis of moderate severity (K05.31) (24 men, 20 women). The condition of periodontal tissues and the intensity of dental caries damage were assessed using the decay/missing/filled index, papillary-marginal-alveolar index, communal periodontal index, determined degree of tooth mobility. Using microbiological methods, biomaterial from the mucous membrane of the prosthetic bed was studied and microbiota composition was determined. Identification and quantification of deoxyribonucleic acid of periodontal disease pathogens was carried out using polymerase chain reaction. Statistical analysis was performed using the Shapiro–Wilk test, Student's t test, and χ^2 test.

RESULTS: The average values of the decay/missing/filled index and the papillary-marginal-alveolar index in patients of the second group were 1.5 and 5.7 times higher, respectively, than in the first. Under chronic generalized periodontitis of moderate severity in the biomaterial statistically significantly more often were found *Neisseria* spp. (7.6 times; p=0.002), *Candida* spp. (1.9 times; p=0.035), *Enterobacterales* (16.2 times; p=0.001), *Enterococcus* spp. (5.0 times; p=0.03) and less often — *Corynebacterium* spp. (10.1 times; p=0.001) and *Streptococcus salivarius* (3.0 times; p=0.001). When patients had partial dentition defects, the identified microorganisms formed stable communities, the nature of the symbiotic relationships in which determined the clinical manifestations of inflammatory changes in the soft periodontal tissues.

CONCLUSION: Increased occurrence of *Candida* spp. and *Enterobacterales* in patients with acquired dentition defects and periodontal inflammation, formation of stable connections between them and antagonistic effect on oral cavity autochthonous is of interest for the development of targeted antimicrobial therapy in preparation for dental orthopedic treatment.

Keywords: periodontium; dental defects; inflammation; microflora of the oral cavity; dentistry.

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Особенности микробиоты ротовой полости у пациентов с малыми и средними дефектами зубных рядов и хроническим пародонтитом средней степени тяжести

О.А. Шулятникова, М.В. Яковлев, А.П. Годовалов

Пермский государственный медицинский университет имени академика Е.А. Вагнера, Пермь, Россия

АННОТАЦИЯ

Обоснование. Хронический генерализованный пародонтит относится к наиболее распространённой патологии зубочелюстной системы и вызывает трудности в принятии рационального плана лечения, поскольку нет биомаркеров для качественной оценки возможности развития воспаления пародонта или его патоморфоза в более тяжёлую стадию.

Цель исследования — изучение особенностей микробиоты ротовой полости у пациентов с приобретёнными дефектами зубных рядов при хроническом генерализованном пародонтите средней степени тяжести.

Материалы и методы. Участниками исследования являлись 88 человек с малыми/средними дефектами зубного ряда (код по Международной классификации болезней 10-го пересмотра — К08.1), которых разделили на две группы: первая — без клинических признаков воспаления тканей пародонта (23 мужчины, 21 женщина); вторая — с хроническим генерализованным пародонтитом средней степени тяжести (К05.31) (24 мужчины, 20 женщин). Для определения стоматологического статуса проводили оценку состояния тканей пародонта и интенсивности поражения зубов кариесом с использованием индексов КПУ (К — число очагов кариеса; П — установленные пломбы; У — удалённые/отсутствующие зубы), папиллярно-маргинально-альвеолярного индекса, коммунального пародонтального индекса, определяли степень подвижности зубов. С применением микробиологических методов исследовали биоматериал со слизистой оболочки тканей протезного ложа и определяли состав микробиоты. Выявление и количественную оценку дезоксирибонуклеиновой кислоты возбудителей заболеваний пародонта проводили с помощью полимеразной цепной реакции. Статистический анализ выполняли с использованием критерия Шапиро–Уилка, t-критерия Стьюдента и критерия χ^2.

Результаты. Средние значения индекса КПУ и папиллярно-маргинально-альвеолярного индекса у пациентов второй группы были соответственно в 1,5 и 5,7 раза больше, чем в первой. У пациентов с хроническим генерализованным пародонтитом средней степени тяжести в биоматериале статистически значимо чаще встречались *Neisseria* spp. (в 7,6 раза; *p*=0,002), *Candida* spp. (в 1,9 раза; *p*=0,035), *Enterobacterales* (в 16,2 раза; *p*=0,001), *Enterococcus* spp. (в 5,0 раза; *p*=0,03) и реже — *Corynebacterium* spp. (в 10,1 раза; *p*=0,001) и *Streptococcus salivarius* (в 3,0 раза; *p*=0,001). При наличии у пациентов частичных дефектов зубных рядов констатированные микроорганизмы формировали стойкие сообщества, характер симбиотических отношений в которых определял клинические проявления воспалительных изменений мягких тканей пародонта.

Заключение. Повышенная встречаемость *Candida* spp. и *Enterobacterales* у пациентов с приобретёнными дефектами зубных рядов и воспалительным процессом мягких тканей пародонта, формирование между ними стойких связей и антагонистическое действие на автохтонные для ротовой полости бактерии представляет интерес для разработки таргетной антимикробной терапии при подготовке к стоматологическому ортопедическому лечению.

Ключевые слова: пародонт; дефекты зубного ряда; воспаление; микрофлора ротовой полости; стоматология.

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ЭКО•ВЕКТОР

BACKGROUND

The prevention of inflammatory diseases of periodontal soft tissues is one of the unsolved problems of modern dentistry. According to various authors, chronic generalized periodontitis is one of the most common dental pathologies [1, 2].

The pathogenesis of acquired defects of the dentition and inflammatory diseases of periodontal soft tissues involves various factors such as odontogenic inflammation, impaired immune status, and dentoalveolar anomalies [3, 4]. In addition to provoking inflammatory processes in periodontal tissues, certain pathologies can significantly reduce the treatment efficiency [5]. The complexity of dental arch defects, secondary deformities such as changes in the position of the teeth, and chronic generalized inflammatory process of periodontal soft tissues cause difficulties in determining a rational treatment plan for orthopedic dentists and dental therapists and affects negatively the procedure for achieving patient compliance [6]. To explain effectively the stages of restoring the integrity of the dental arches to patients who have sought dental orthopedic treatment, the need for preliminary professional oral hygiene and treatment of periodontal soft tissue inflammation and the cause of this disease induced by opportunistic microorganisms in the oral cavity must be informatively demonstrated and explained [7].

Microorganisms attach both to the open surfaces of their oral structures and materials of dental orthopedic structures and form a biofilm through cooperation and complex interaction, which increases their virulence and resistance [8]. Such spatiostructural association of individual strains of microorganisms existing in the extracellular polysaccharide matrix is the main factor in the emergence of an overwhelming range of pathological processes, including inflammatory ones [9, 10].

Numerous studies [9, 11, 12] have confirmed the importance of not only the bacterial composition of biofilms formed on the surface of hard tissues of teeth and elements of dental structures but also the amount of plaque, as well as its duration of direct contact with the soft tissues of the considered biotope, in the development of inflammatory pathologies of periodontal the soft tissues. Previously, researchers [13–15] have confirmed the predominance of Gram-positive aerobic microorganisms in a healthy periodontium, with the possible presence of 10–15% of Gram-negative microorganisms. However, inflammatory diseases of this biotope are characterized by a change in this ratio to the opposite, depending on the severity of the inflammatory process. To date, out of 400–500 species of microorganisms, 10–15 periodontopathogens were identified as specific to the oral cavity [16]. Another group of authors who examined the oral microbiota [17, 18] established the frequency of the occurrence of five main opportunistic microorganisms, which are inflammatory markers in periodontal tissues, not exceeding 6.6%, namely, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsytus*, *Actinobacillus actinomycetemcomitans*, and *Treponema denticola*.

Thus, to date, even with the above-mentioned informative research results, biological markers for the qualitative assessment of the risk for inflammation in periodontal soft tissues or its pathomorphosis in a more severe stage have not been identified.

This study aimed to investigate the characteristics of the oral microbiota in patients with acquired dental defects and moderate chronic generalized periodontitis.

MATERIALS AND METHODS

Research design

An observational, multicenter, cross-sectional, sampling, uncontrolled study was conducted.

Eligibility criteria

The study included 88 patients (44 men and 44 women) aged 60–74 years with minor (missing 1–3 teeth) and moderate (missing 4–6 teeth) dental defects [International Classification of Diseases, 10th revision (ICD-10) code K08.1], who had not previously used removable dentures.

The inclusion criteria were as follows: availability of informed, voluntary consent to participate in the study and use of medical data for scientific purposes; absence of bad habits (alcohol, nicotine and drug addiction); nonuse of antibiotics or antiseptics within 3 months before the study; diagnosis of partial absence of teeth on both jaws (ICD-10 K08.1); diagnosis of chronic generalized moderate periodontitis (ICD-10 K05.31) (second group); absence of neoplasms; absence of previously installed dental implants; and last tooth extraction >1 year before the present examination.

The exclusion criteria were as follows: bad habits (alcohol, nicotine, and drug addiction); intake of antibiotics or use of antiseptics within 3 months before the examination; presence of neoplasms; general somatic pathology in the acute stage; previous installation of dental implants; and last tooth extraction <6 months before the present study.

Voluntary refusal to participate in the study was a criterion for exclusion from the observation group.

Study conditions

The study was conducted at the City Dental Clinic of the Perm Territory and the dental clinic of the Clinical Multidisciplinary Medical Center of the E.A. Wagner Perm State Medical University. In the set of clinical dental examinations, both groups underwent X-ray (orthopantomography) imaging at the "3D Diagnostics" and "3D StomatoLorica" (Perm).

Study duration

The study was conducted for 14 months (from October 1, 2021, to November 28, 2022) and included a comprehensive

dental examination of patients in observation groups with minor and moderate dental defects (K08.1). Biomaterials from the oral cavity were collected as an imprint smear for microbiological studies.

Medical intervention

To assess the dental status and achieve clarity in the study, an index assessment of the periodontal tissue condition was performed using standard methods (papillary–marginal–alveolar index and community periodontal index). The degree of tooth mobility was recorded according to the Miller scale (S.C. Miller, 1938) as modified by Flezar (T.J. Flezar et al., 1980) during dental examination, and the intensity index of dental caries damage was detected as CFR (where C is the number of caries foci; F is installed fillings; R is removed/missing teeth) based on orthopantomogram data (hidden defects of hard dental tissues).

In the observation groups, biomaterials were collected from the central part of the apex (alveolar process/alveolar part) of the dental defect area using swab probes. After preliminary dilution (1:1,000), the contents were inoculated on blood agar, Endo and Sabouraud media, and selective media for isolating streptococci. Incubation was performed at a temperature of 37 °C in a humid atmosphere under microaerophilic conditions. The isolated strains were identified by cultural, tinctorial, and biochemical characteristics.

Detection and quantitative assessment of DNA of periodontal pathogens were performed using the "DENTOSCREEN" reagent kit (Scientific and Production Company "LITEKH", Russia) by the polymerase chain reaction with hybridization-fluorescence detection in real time using a CFX96 Touch amplifier ("Bio-Rad", USA).

Main study outcome

The presence of oral microbiota representatives was recorded in patients with minor and moderate acquired dental defects in the presence of chronic inflammatory pathology of periodontal soft tissues.

Additional study outcomes

The severity of interactions between members of the oral microbiocenosis was established in patients with minor and moderate acquired dental defects depending on the clinical signs of periodontal tissue inflammation.

Subgroup analysis

Depending on the clinical signs of the periodontal soft tissue inflammation, the patients were distributed into two groups, where first group included patients with minor and moderate dental defects (K08.1) without signs of periodontal soft tissue inflammation, and second group consisted of patients with minor and moderate dental defects (K08.1) and moderate chronic generalized periodontitis (K05.31).

Methods of registration of outcomes

The proportion of different microorganisms in the oral microbiota was assessed using the coefficient of constancy of the species, which was calculated using the following equation:

$$C=p\times100/P,$$

where p is the number of cases with the registration of the species studied and P is the total number of cases.

To quantify the interaction between the microbiocenosis members, the Jaccard similarity coefficient was calculated using the following equation:

$$q=c/(a+b-c)\times100,$$

where a is the number of cases with species a, b is the number of cases with species b, and c is the number of cases containing both species.

The nature of the relationship between the two species in the microbiota was determined by the ratio $P1$:$C1$, where $P1$ is the probable number of cases where two randomly selected species exist together and $C1$ is the number of cases containing both species.

Ethical considerations

The clinical and experimental studies were approved at a meeting of the local ethics committee of the E.A. Wagner Perm State Medical University (Protocol No. 9 dated September 30, 2021).

Statistical analysis

Statistical data processing was performed using Statistica 7.0. The Shapiro–Wilk test was used to test the normality of the distribution. If the distribution was close to normal, the Student t-test was used; in other cases, the Mann–Whitney test was used to assess the significance of differences. The results are presented as the mean and its error (M±m). The significance of the differences between the indicators was assessed by nonparametric analysis of the χ^2 criterion.

RESULTS

Research objects (participants)

The study participants were individuals aged 60–74 years (n=88, with 44 men and 44 women) with minor and moderate dental defects (K08.1), who had not previously used removable dentures. First group (n=44, with 23 men and 21 women) included patients with minor and moderate dental defects without signs of periodontal soft tissue inflammation. Second group included 44 patients (24 men and 20 women) with minor and moderate dental defects and a verified diagnosis of moderate chronic generalized periodontitis (K05.31).

Main study outcomes

Table 1 presents the results of the clinical assessment of the periodontal soft tissues and hard dental tissues of the observation groups.

Table 1. Index assessment of periodontal tissues and dental hard tissues in patients of observation groups (*n*=88)

Index	First group (*n*=44)	Second group (*n*=44)	*p*
PMA (%)	11.7±2.0	54.1±5.8	0.001
CPI	0.2±0.1	4.1±0.3	0.001
DMF	12.6±1.0	17.1±1.2	0.005

Note. Student's t-test was used for statistical evaluation of data. PMA — papillary-marginal-alveolar Index. CPI — communal periodontal index. DMF — decay/missing/filled index.

The assessment of the condition of the periodontal tissues in the observation groups using the papillary–marginal–alveolar index indicated the presence of moderate gingivitis of (54.1±5.8)% in second group. This indicator differed significantly from that of first group, which had a value of (11.7±2.0)% and corresponded to mild gingivitis. In first group, interdental papilla inflammation was not visually determined; however, it was recorded during the Schiller–Pisarev test on 1–2 teeth, which was most often associated with an unsatisfactory condition of fillings (overhanging edge) or caries in the gingival margin of the examined teeth.

A similar presentation was noted when assessing the condition of periodontal tissues using the community periodontal index. Thus, in some patients of the first group, signs of periodontal tissue inflammation were not visually determined; however, during examination using a periodontal probe, isolated signs of the initial stage of inflammation were detected in one of the sextants in the form of bleeding gums during probing. In second group, a periodontal pocket with a depth of up to 4–5 mm was detected in 43.2% of the patients (*n*=19) and that with a depth of 6 mm was registered in 56.8% (*n*=25) (Fig. 1). Table 1 indicates that the clinical signs of periodontal tissue damage are significantly different between the groups.

When assessing the average caries intensity index of the CFR index, the average caries intensity in second group was significantly very high in comparison with that in first group, with 17.1±1.2 and 12.6±1.0, respectively.

In second group, grade I tooth mobility was established in 38.6% of the patients (*n*=17), whereas grade II was identified in 44.4% (*n*=20). Mobility within the physiological norm was registered in 15.9% of the cases (*n*=7). In first group, grade I tooth mobility was diagnosed only in nine patients (20.5%).

Patients with minor and moderate defects of the dental arches demonstrated significant differences in the biofilm microbiota composition depending on the presence or absence of periodontal soft tissue inflammation (Table 2). Thus, in patients diagnosed with moderate chronic generalized periodontitis (K05.31), streptococci species that are provoking factors in the development of inflammatory diseases were naturally more common. Moreover, a rarer (>3 times) representation of Streptococcus salivarius in second group indicates an antagonistic effect of other microorganisms on this species. In addition, opportunistic Neisseria (34.1%;

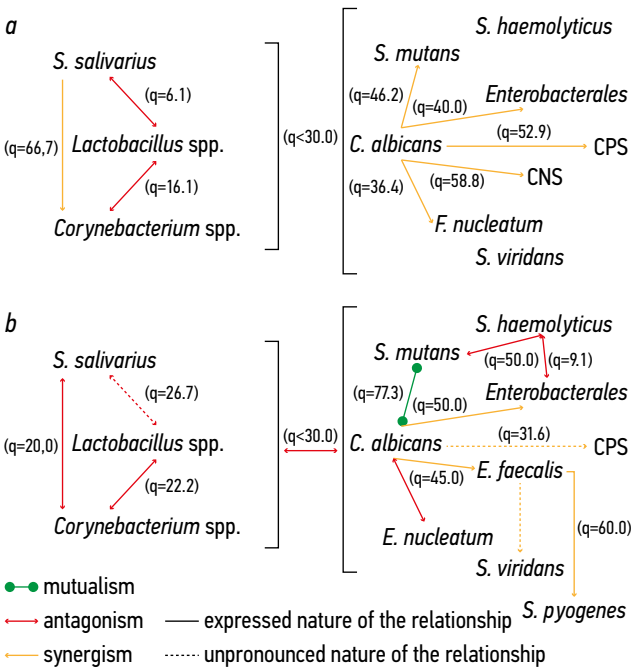

Fig. 1. Nature of symbiotic relationships of oral microorganisms of patients: *a* — first group; *b* — second group. CPS — coagulase-positive staphylococci, CNS — coagulase-negative staphylococci, q — Jaccard similarity coefficient.

p=0.002) was predominant in patients with moderate chronic generalized periodontitis compared with individuals with minor and moderate dental defects without clinical signs of periodontal soft tissue inflammation, who have not previously used dentures. In addition, the incidence of Gram-positive rod-shaped microorganisms (*Corynebacterium* spp. is detected >10 times less frequently) decreased in second group.

Additional study results

Depending on the presence of clinical signs of periodontal soft tissue inflammation, microecological variants of the oral microbiota were established. In each of them, two stable groups of microorganisms were identified. First group included *S. salivarius*, *Lactobacillus* spp., and *Corynebacterium* spp., which are autochthonous bacteria in the oral cavity (microorganisms typical for the oral cavity). Opportunistic allochthonous microbes (microorganisms inherent in other areas of the body, e.g., the intestines or nasopharynx), such as *Candida albicans* and *Enterobacterales*, constituted second group. Moreover, changes in symbiotic relationships were registered between and within the communities.

In patients without clinical signs of periodontal soft tissue inflammation, antagonistic relationships were detected between allochthonous and autochthonous microorganisms (see Fig. 1). In addition, synergism with *C. albicans* of most isolated strains of opportunistic bacteria was characteristic. As regards the degree of incompatibility, *S. salivarius* and *Staphylococcus haemolyticus*, as well as *S. salivarius* and *Lactobacillus* spp., demonstrated the highest level of incompatibility.

The antagonistic relationships in patients with minor dental defects (who had not previously worn dentures and had no

Table 2. Frequency of occurrence of certain types of microorganisms in smears obtained from the surface of the oral mucosa in patients of the observation groups (*n*=88) (% of cases)

Microorganism	First group (*n*=44)	Second group (*n*=44)	*p*
Staphylococcus spp.:	97.7	93.2	0.31
Coagulase-negative	38.6	17	0.001
Coagulase-positive	36.4	13.6	0.027
S. aureus	100	100	—
S. intermedius	0	0	—
S. hyicus	0	0	—
Streptococcus spp.:	100	56.8	0.7
S. salivarius	68.2	22.7	0.001
S. oralis	43.2	20.5	0.04
S. pyogenes	4.5	13.6	0.27
Neisseria spp.	4.5	34.1	0.002
Candida spp.:	22.7	43.2	0.042
C. albicans	50.0	68.4	0.035
Enterobacterales:	4.5	72.7	0.001
E. coli	100	87.5	0.001
Klebsiella spp.	0	9.4	0.6
Enterobacter spp.	0	3.1	0.5
Lactobacillus spp.	13.6	20.5	0.6
Corynebacterium spp.	45.5	4.5	0.001
Enterococcus spp.	4.5	22.7	0.03
Fusobacterium nucleatum	11.4	25.0	0.09
Treponema denticola	0	4.5	0.5

Note. χ^2-criterion was used for statistical evaluation of data.

clinical signs of periodontal soft tissue inflammation) not only persist with a clinically and radiologically verified diagnosis of moderate chronic generalized periodontitis, but, as shown in this study, are enhanced by additional pressure from *Enterococcus faecalis* and *Streptococcus pyogenes*. As a result, the listed factors lead to a change in the composition of the permanent microflora of the oral cavity. The enhancement of antagonistic relationships within the community of autochthonous microorganisms in patients is associated with oral mucosa inflammation. On the contrary, in the individuals examined, with the development of chronic generalized periodontitis, synergistic relationships among allochthonous microbes are enhanced, up to the development of mutualism between *C. albicans* and *Streptococcus mutans*. Moreover, autochthonous bacteria lose their probiotic properties, which are pronounced in the loss of their effect on *S. mutans* and *S. haemolyticus*.

Adverse events

No adverse events were registered.

DISCUSSION

Summary of the main study outcome

The average CFR and papillary–marginal–alveolar indexes in second group were 1.5 and 5.7 times higher, respectively, than those in first group. In addition, the incidence of moderate chronic generalized periodontitis (ICD-10 K05.31) was significantly higher with *Neisseria* spp. (7.6 times; *p*=0.002), *Candida* spp. (1.9 times; *p*=0.035), *Enterobacterales* (16.2 times; *p*=0.001), and *Enterococcus* spp. (5.0 times; *p*=0.03) and significantly lower with *Corynebacterium* spp. (10.1 times; *p*=0.001) and *S. salivarius* (3.0 times; *p*=0.001).

Discussion of the main study outcome

In the examined individuals with minor and moderate dental defects, a significant difference was revealed in the index assessment of the condition of the periodontal soft tissues and intensity of dental caries damage in groups with visually undetectable or absent clinical signs of inflammation and a verified diagnosis of chronic generalized moderate periodontitis (K05.31).

The revealed synergism of the isolated strains of opportunistic bacteria with *C. albicans* is of interest for the targeted antimycotic preventive action at diagnosis and preparation for orthopedic treatment of patients with minor and moderate dental defects.

Considering the information on the production of bioactive metabolites by lactobacilli that promote the regression of dysbiosis of the oral cavity and periodontitis therapy [19], the high level of paired incompatibility of *S. salivarius*

and *S. haemolyticus*, and *S. salivarius* and *Lactobacillus* spp., is of interest for the targeted antibacterial action on *S. salivarius* in the development of secondary periodontal soft tissue inflammation.

Study limitations

Given the peculiarities of the bacteriological method, one of the limitations may be the insufficient registration of noncultivated species of microorganisms and strains that have converted to L-forms under the influence of antibiotics.

CONCLUSION

The increased abundance of Candida spp. and Enterobacterales in patients with dental defects and periodontal soft tissue inflammation, formation of stable relationships by these microorganisms, and antagonistic effect on autochthonous oral bacteria, such as *Corynebacterium* spp., are important in the development of not only targeted antimicrobial therapy when planning orthopedic treatment but also methods for express diagnostics of marker microorganisms for the early prevention of chronic generalized periodontitis.

ADDITIONAL INFORMATION

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Authors' contribution. O.A. Shuliatnikova — oversaw the project, designed the study, analyzed data, wrote the manuscript with input from all authors; A.P. Godovalov — designed the study, analyzed data, wrote the manuscript with input from all authors; M.V. Yakovlev — examination of patients, designed the study, analyzed data, wrote the manuscript with input from all authors. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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AUTHORS' INFO

*** Anatoliy P. Godovalov,** MD, Cand. Sci. (Medicine), Associate Professor;
address: 26 Petropavlovskaja street, 614990 Perm, Russia;
ORCID: 0000-0002-5112-2003;
eLibrary SPIN: 4482-4378;
e-mail: agodovalov@gmail.com

Oksana A. Shuliatnikova, MD, Dr. Sci. (Medicine), Associate Professor;
ORCID: 0000-0002-2033-5903;
eLibrary SPIN: 4670-4605;
e-mail: anasko06@mail.ru

Mikhail V. Yakovlev, MD, Cand. Sci. (Medicine);
ORCID: 0000-0002-2895-387X;
eLibrary SPIN: 4665-2340;
e-mail: mikhailyak@mail.ru

ОБ АВТОРАХ

*** Годовалов Анатолий Петрович,** канд. мед. наук, доцент;
адрес: Россия, 614990, Пермь, ул. Петропавловская, д. 26;
ORCID: 0000-0002-5112-2003;
eLibrary SPIN: 4482-4378;
e-mail: agodovalov@gmail.com

Шулятникова Оксана Александровна, д-р мед. наук, доцент;
ORCID: 0000-0002-2033-5903;
eLibrary SPIN: 4670-4605;
e-mail: anasko06@mail.ru

Яковлев Михаил Владимирович, канд. мед. наук;
ORCID: 0000-0002-2895-387X;
eLibrary SPIN: 4665-2340;
e-mail: mikhailyak@mail.ru

* Corresponding author / Автор, ответственный за переписку