GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 3 (360) Март 2025

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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Содержание:

GEORGIAN MEDICAL NEWS
No 3 (360) 2025

ANALYSIS OF PERIODONTAL POCKET MICROBIOTA IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS

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Abstract.

Introduction: Periodontal diseases are considered to be among the most prevalent diseases in the maxillofacial region. As indicated by contemporary data, the etiology of inflammatory periodontal diseases is attributed to the accumulation of microbial biofilms on the surfaces of teeth in close proximity to periodontal tissues. The increased activity of periodontopathogenic bacteria can lead to the activation of the body's immune response cascade, which can contribute to the development of gingivitis and periodontitis. The objective of the present study was to investigate the composition of the microbial association of the periodontal pocket in patients exhibiting varying degrees of periodontal tissue damage.

Materials and Methods: The microbiologic composition of periodontal pocket contents was studied in a cohort of 34 patients diagnosed with K05.3 Chronic periodontitis. The state of periodontal tissues was evaluated using clinical, instrumental, and radiologic examination methods. The contents of the periodontal pocket were utilized as a subject for examination. The primary identification of microorganisms was carried out by means of the MALDI TOF MS method.

Results: The results of the study indicated the presence of various indicators of microbial composition in patients exhibiting different degrees of periodontal tissue lesions. The contents of the periodontal pocket were found to contain both aerobic and anaerobic representatives of bacterial flora. Furthermore, the presence of representatives from the periodontopathogenic spectrum of microorganisms was detected.

Conclusions: The microbial composition of the contents of periodontal pockets varies according to the extent of damage to periodontal structures. As the severity of the disease worsened, there was a shift towards an increase in the number of obligate microorganisms and a decrease in species diversity.

Key words. Marginal periodontitis, periodontal pocket, microflora, CFU, polyhexanide.

Introduction.

Diseases of marginal periodontal tissues represent a pressing challenge for contemporary dentistry. Inflammatory diseases of periodontal tissues account for the majority of diseases affecting soft tissues in the region surrounding teeth. The primary nosologic units are gingivitis and periodontitis. Gingivitis is defined as an inflammation of the gingival tissues without destruction of the gingival attachment and without changes in the bone tissue. Inflammation is typically localized to the area of free and partially attached gingiva [1,2]. The exacerbation and subsequent progression of the inflammatory response may result in the onset of marginal periodontitis. Such inflammation encompasses a greater volume of soft tissue, resulting in the destruction of epithelial attachment and bone tissue. This process ultimately leads to the formation of periodontal pocket [3,4].

Contemporary data indicates that the etiopathogenetic basis for the development of inflammatory periodontal diseases is the formation of microbial biofilm on the surface of teeth in close proximity to periodontal tissues. The mechanisms of the macro-organism's response to the pathogenic influence of bacterial flora also play an important role. A variety of factors present within the oral cavity have the capacity to contribute to alterations in the biocenosis of the oral cavity, thereby resulting in an imbalance in the ratio of certain representatives of opportunistic flora. The increased activity of periodontopathogenic bacteria can lead to the activation of the body's immune response cascade, which can contribute to the development of gingivitis and periodontitis. It has been established that *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium nucleatum*, and a number of other microorganisms play a pivotal role in the development of inflammatory periodontal diseases. It has been demonstrated that periodontopathogenic flora is capable of exerting the most deleterious effects on periodontal tissues due to the presence of specific pathogenicity factors [5,6].

The factors that increase the virulence of periodontopathogens include the following. Firstly, there is the ability to adhere to gingival cells with subsequent colonization. Secondly, there is the ability to destroy epithelial cell barriers. Lastly, there is the increased secretion activity of destructive enzymes, which includes elastase, collagenase, phospholipase, and trypsin- and chymotrypsin-like proteases. In addition, there are mechanisms characteristic of specific bacterial species that contribute to the destruction of periodontal tissues and suppression of local immune defense reactions [7]. Mechanisms present in *Porphyromonas gingivalis* include the following: the presence of fimbriae, the synthesis of gingipain, and the production of endotoxin—lipopolysaccharide of the cell wall. In *Actinobacillus actinomycetemcomitans*, there is secretion of exotoxin—leukotoxin. *Tannerella forsythia* is characterized by the production of glyco- and proteolytic enzymes, as well as the ability to trigger the process of apoptosis in the cells of periodontal tissues [8,9].

The presence of a large number of pathogenicity factors of opportunistic flora in the oral cavity and their increased activity under favorable conditions pose significant risks of inflammatory diseases of the periodontal tissues. Numerous studies have demonstrated a correlation between the presence of inflammatory periodontal diseases and the development or exacerbation of the course of general medical diseases [10,11]. There is also evidence indicative of a correlation between inflammation of periodontal structures and complications in the

course or termination of pregnancy [12]. This substantiates the paramount significance of understanding the composition of periodontopathogenic flora and the necessity of incorporating this knowledge into the treatment planning process for inflammatory diseases of the periodontal tissues.

The objective of this study was to examine the composition of the microbial association of the periodontal pocket in patients with varying degrees of periodontal tissue lesions.

Materials and Methods.

Sampling:

The microbiological composition of the periodontal pocket contents was examined in a cohort of 34 patients diagnosed with K05.3 Chronic periodontitis. The state of periodontal tissues was assessed using clinical, instrumental, and radiologic examination methods. The visual parameters of the periodontal tissues were evaluated by assessing changes in gingival color (presence of hyperemia, cyanosis), palpation of the gingiva, and the presence of pathological elements (ulcers, erosions, necrotic changes, etc.). The gingival sulcus was probed with a graduated periodontal probe to ascertain the level of attached epithelium, the presence of bleeding, and the presence and depth of periodontal pockets in six points. The purpose of the orthopantomogram was to study the state of the cortical lamina of interdental septa, the presence of atrophy, resorption, and osteoporosis of bone tissue, the state of furcation areas, and the presence of bone pockets.

The age of the patients included in the study ranged from 20 to 60 years.

The prevalence of periodontitis was found to vary, with 26% of subjects exhibiting mild symptoms, 39% displaying moderate symptoms, and 35% manifesting severe symptoms.

The inclusion and exclusion criteria employed are delineated in Table 1.

The patients were divided into three groups:

Group 1: Patients with mild chronic generalized periodontitis (n = 9).

Group 2: Patients with moderate chronic generalized periodontitis (n = 13).

Group 3: Patients with severe chronic generalized periodontitis (n =12).

The material for the study was obtained from the contents of the periodontal pocket, which was collected under the supervision of Central Research Institute of Dentistry and Maxillofacial Surgery. Samples for microbiological study were obtained directly from the periodontal pocket while ensuring isolation from saliva by using cotton rolls. Sterile paper pins (absorbers) of size #30 by ISO, were placed to the full depth of the periodontal pocket in the amount of 3 pieces for 10 seconds for material collection. The prepared samples were then aseptically placed in sterile tubes containing thioglycol medium.

The severity of chronic periodontitis was classified based on clinical attachment loss (CAL) and radiographic bone loss.

- **Mild:** CAL 1–2 mm, bone loss <15% of root length.
- **Moderate:** CAL 3–4 mm, bone loss 15–33%.
- **Severe:** CAL ≥5 mm, bone loss >33% and/or tooth mobility Grade II or higher.

These criteria follow the classification proposed by the American Academy of Periodontology (2018).

Extraction of pure cultures:

The isolation of anaerobic microorganisms was performed by inoculation on Schaedler's anaerobic agar (Oxoid, Basingstoke; UK) with 5% (v/v) defibrinated sheep blood, anaerobic basal agar (Oxoid, Basingstoke; UK) with defibrinated sheep blood, or Lactobacillus MRS Agar (Himedia Labs. Inc., India) with defibrinated sheep blood. The following media were utilized for the isolation of aerobic microorganisms: The following agar media are employed: Endo Agar (Becton Dickinson and Company, USA), gelatin-mannitol salt agar (Staphylococcus Agar #110, Himedia Labs Inc., India), and m-Enterococcus Agar (Difco Laboratories, Franklin Lakes, USA). Serial dilutions of periodontal pocket contents in Columbia Broth liquid medium (Himedia Labs Inc., Mumbai, India) were prepared for media inoculation. The inoculation process was executed in 10^2, 10^3, 10^5, and 10^7 dilutions.

Following the inoculation of anaerobic bacteria, the Petri dishes were placed into anaerobic jars (Schutt Labortechnik GmbH, Göttingen, Germany) containing a gas mixture (85% N_2, 10% H_2, 5% CO_2) in the presence of platinum catalysts at 37°C for a period of 72 hours.

Petri dishes inoculated with the test organism were examined macroscopically at the conclusion of the incubation period for evidence of colony growth. The dishes were then subjected to a morphological classification, and the number of each type of colony was recorded. The subsequent step entailed a microscopic examination of the colonies, accompanied by preliminary Gram staining.

The colonies that were identified were separated and transferred to separate Petri dishes with the same medium. Then, they were incubated under anaerobic or aerobic conditions to obtain bacterial biomass for further identification and preservation. In the present study, a particular strain of identified microorganisms was subjected to a freeze-drying process. This procedure entailed the microbial suspension being frozen in a cryoprotectant solution (10% sucrose/1% gelatin [w/v]), which was subsequently processed using a freeze dryer (SB1, Chemlab, UK) to ensure its prolonged preservation. The storage of tubes containing dried microbial strains was conducted at a temperature of -80°C.

The quantification of bacterial load was performed using a colony-forming unit (CFU) assay. Transportation of the collected samples of the test material to the laboratory was conducted by placing paper pins in a test tube with 5 milliliters of thioglycol transport medium. The sample mass was determined by subtracting the mass of the empty container from the mass of the container with dried sample. All samples were subjected to culturing for a period of two hours following their collection. Following the process of homogenization, 10-fold dilutions of 10^2–10^7 were prepared in Columbia Broth liquid medium (Himedia Labs Inc., Mumbai, India). From the respective dilutions, 0.1 ml samples were seeded on selective and non-selective media. The bacterial counts were expressed as log10 colony-forming units per 1 g of sample (log10 CFU/g).

MALDI-TOF MS and 16s rRNA Sequencing:

The primary identification of microorganisms was performed by MALDI TOF MS on a Vitek MS Plus instrument according to the recommendations of the equipment manufacturer. In instances where the species of bacterial strains could not be identified by mass spectrometry, identification was facilitated by sequencing of the 16S rRNA gene fragment. For this purpose, the polymerase chain reaction (PCR) was utilized to amplify the 16S rRNA gene fragment using universal bacterial primers 27F (5′-AGAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-ACGGGGYTACCTTTGTTGTTACGACTT-3′) for 35 cycles, employing the subsequent program: The denaturation process was conducted at a temperature of 94°C for 20 seconds. Subsequently, the primer annealing phase was initiated at 58°C for an equal duration. The elongation stage was then carried out at 72°C for a period of 90 seconds. The resulting PCR product was purified using the Cleanup Standard kit (Evrogen, Moscow, Russian Federation). The amplification of the DNA fragment was followed by Sanger sequencing with primer UF1 at Evrogen (Moscow, Russian Federation). The sequence cutoff boundaries were determined based on the quality of electrophoregrams visually using the Chromas Lite program, version 2.6.6 (Technelysium Pty. Ltd., Australia). The identification of bacterial species was facilitated by conducting a search of the retrieved nucleotide sequences in the GenBank database using the Megablast algorithm. The comparison result was deemed species-level appropriate if its partially sequenced 16S rRNA gene sequence exhibited a minimum of 98.7% similarity to the sequence of the nearest known bacterial species in the GenBank database.

Statistical Analysis:

The statistical processing of the data was performed using Excel and XLSTAT programs, which were developed by Addinsof Inc. (New York, NY, USA). The R statistical programming language was utilized to conduct the data analysis. The illustrations were plotted using the ggplot2 package. In the course of all statistical analyses, a value of $P \leq 0.05$ was considered to be significant.

Results.

The results of the study demonstrated the presence of various indicators of microbial composition among patients exhibiting differing degrees of periodontal tissue damage. The presence of both aerobic and anaerobic representatives of bacterial flora was detected in the contents of the periodontal pocket. Furthermore, the presence of periodontopathogenic microorganisms was detected.

Table 2 provides a synopsis of the identified bacteria, accompanied by their phylotype, family, and species classification. In this study, the sample mean is denoted as M, and the standard error is denoted as m. A total of 24 bacterial species were cultivated, 12 of which were Gram positive and 12 of which were Gram negative. The diversity of bacteria according to the type of oxygen utilization was observed, and facultative and obligate anaerobes were identified. Morphological analysis revealed a predominance of bacilli, accompanied by the presence of cocci. Furthermore, the presence of fungal flora was detected.

The mean degree of infestation in subjects with mild periodontal tissue damage was 4.7 log10 CFU/g ± 0.15 log10 CFU/g, with a range from 2.1 ± 0.08 log10 CFU/g (*Eikenella corrodens*) to 8.6 ± 0.12 log10 CFU/g (*Veillonella spp.*). The mean degree of infestation in patients with moderate periodontal tissue damage was 4.9 log10 CFU/g ± 0.15 log10 CFU/g, ranging from 0.5 ± 0.12 log10 CFU/g (*Aggregatibacter actinomycetemcomitans*) to 8.5 ± 0.18 log10 CFU/g (*Actinomyces odontolyticus*).

The mean degree of infestation in patients with severe periodontal tissue damage was determined to be 4.8 log10 CFU/g ± 0.16 log10 CFU/g, with a range from 0.3 ± 0.20 log10 CFU/g (*Aggregatibacter actinomycetemcomitans*) to 8.7 ± 0.11 log10 CFU/g (*Porphyromonas gingivalis*) (Figure 1).

The results of the study revealed that Group 1, which comprised patients with mild severity of periodontal tissue lesions, predominantly exhibited bacilliform bacteria of both anaerobic and aerobic types. In the context of gram-positive bacteria of anaerobic and facultatively anaerobic nature, coccal flora, typified by streptococci, manifested as the predominant bacterial type. Gram-negative anaerobic flora is represented to a greater extent by *Veillonella, Fusobacteria*, and *Bacteroides*, which are included in the list of the main periodontopathogens (*Porphyromonas gingivalis, Tannerella forsythia*, and *Prevotella intermedia*). Furthermore, the presence of bacteria belonging to the gram-positive anaerobic flora, predominantly comprising actinomycetes, was also identified. Furthermore, the presence of bacteria that are not common representatives of the resident flora of the oral cavity (*Enterococcus faecalis, Enterobacter cloacae, Escherichia coli*) was also detected, indicating the development of dysbiosis. Furthermore, the presence of fungi belonging to the genus Candida was detected.

The microbial composition of periodontal pocket contents in Group 2 patients with an average degree of periodontal structures lesion exhibited differences. A decline in the prevalence of gram-positive cocci and lactobacilli was observed. A significant increase in the prevalence of gram-negative bacilliform flora was observed in comparison with the group of patients exhibiting mild disease severity. The

Table 1. *The inclusion and exclusion criteria.*

Inclusion criteria	***Exclusion criteria***
1. Age ranging from 20 to 60 years. 2. Diagnosed marginal periodontitis clinically and radiologically confirmed. 3. Informed consent to participate in the study (protocol of the local ethical committee of PFUR Medical Institute No. 13 dated December 15, 2022).	1. Presence of acute generalized diseases. 2. Presence of infectious diseases (except periodontitis). 3. Presence of pregnancy at the time of the study. 4. Age less than 20 and more than 60 years. 5. Antibacterial medication in the anamnesis during 6 months before the beginning of the study. 6. Visiting a dentist for periodontitis treatment in the last year before the study. 7. Refusal to participate in the study at any stage.

Table 2. *Microbiome of periodontal pockets in patients with different degrees of periodontal tissue lesion. ll microbial names are italicized. Percentages indicate the detection frequency of each microorganism among the analyzed samples.*

Phylotype	Species	Group 1		Group 2		Group 3	
		%	*log10 CFU/g (M ± m)*	%	*log10 CFU/g (M ± m)*	%	*log10 CFU/g (M ± m)*
Bacillota	Lactobacillus spp.	76 %	4,5± 0,18	58 %	2,9± 0,10	-	-
	Streptococcus salivarius	83 %	6,3± 0,11	67 %	7,4± 0,16	10 %	0,7± 0,08
	Streptococcus oralis	-	-	12 %	4,4± 0,11	40 %	6,0± 0,21
	Streptococcus sanguinis	93 %	6,8± 0,19	77 %	5,1± 0,18	35 %	3,3± 0,10
	Streptococcus itermedius	88 %	7,6± 0,20	52 %	2,6± 0,11	-	-
	Staphylococcus aureus	-	-	21 %	4,2± 0,18	48 %	6,0± 0,16
	Veillonella spp.	72 %	8,6± 0,12	58,3 %	6,1± 0,20	10,2 %	-
	Enterococcus faecalis	44 %	4,7±0,18	57 %	5,0± 0,14	67 %	6,0± 0,11
	Peptostreptococcus stomatis	15 %	4,4± 0,19	41 %	6,6± 0,20	55 %	5,6± 0,10
Actinomycetota	Actinomyces odontolyticus	-	-	49 %	8,5± 0,18	-	-
	Actinomyces naeslundii	30 %	4,3± 0,12	66 %	7,5± 0,20	27 %	3,0± 0,17
	Actinomyces viscosus	-	-	33 %	2,8± 0,21	-	-
	Actinomyces israelii	43 %	4,0± 0,16	39 %	2,4± 0,19	16 %	1,2± 0,30
Bacteroidota	Capnocytophaga gingivalis	23 %	2,2± 0,21	41 %	4,5± 0,17	42 %	4,0± 0,18
	Porphyromonas gingivalis	67 %	4,5± 0,14	78 %	8,5± 0,16	93 %	8,7± 0,11
	Tannerella forsythia	53 %	5,3± 0,16	70 %	4,8± 0,12	77 %	5,1± 0,12
	Prevotella intermedia	48 %	3,5± 0,17	59 %	2,7± 0,09	73 %	5,6± 0,16
	Prevotella oralis	30 %	5,0± 0,11	26 %	2,6± 0,09	-	-
Fusobacteriota	Fusobacterium nucleatum	100 %	7,1± 0,10	100 %	8,0± 0,11	98,3 %	6,3± 0,21
Pseudomonadota	Eikenella corrodens	40 %	2,1± 0,08	-	-	-	-
	Aggregatibacter actinomycetemcomitans	5 %	4,6± 0,12	2 %	0,5± 0,12	3 %	0,3± 0,20
	Enterobacter cloacae	4 %	3,0± 0,21	15 %	7,4± 0,16	18 %	6,1± 0,21
	Escherichia coli	28 %	2,7± 0,11	34 %	5,1± 0,19	38 %	7,6± 0,19
Ascomycota	Candida albicans	44 %	3,2± 0,14	41 %	4,6± 0,18	75 %	7,0± 0,10

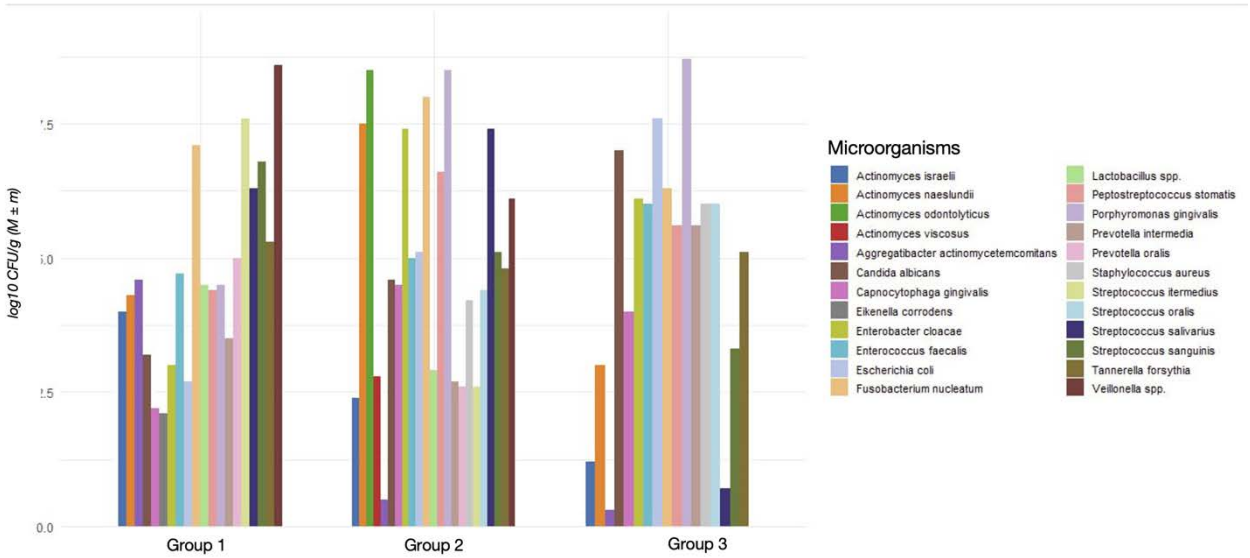

Figure 1. *Composition of periodontal pocket microflora in patients with different degrees of periodontal tissue damage.*

detection percentage of gram-negative anaerobic flora exhibited a significant increase. A substantial increase was also observed in the number of *Actinomyces naeslundii*, with a mean of 7.5 ± 0.20 log10 CFU/g, and a concomitant increase in the diversity of *Actinomycetes*. The number of *Enterobacteriaceae* and fungi increased in comparison with mild severity, indicating a worsening of dysbiosis and displacement of the resident flora.

A decline in species diversity of the flora was identified in Group 3, which comprised patients with severe periodontitis. The number of *streptococci* was found to be significantly reduced, while *lactobacilli* were not detected. However, a high percentage of *Staphylococcus aureus* was isolated, with a mean of 6.0 ± 0.16 log10 CFU/g. Among the gram-negative anaerobic flora, *Porphyromonas gingivalis* was predominant (93% of samples), and the prevalence of *Veillonella* decreased significantly (10% of samples). The number of Fusobacteria remained at a high level. The number of Gram-positive anaerobic flora representatives decreased significantly in comparison with the samples from group 2. The presence of elevated levels of *Enterobacteriaceae* and the fungal genus *Candida* suggests a significant displacement of the native microbiota and the subsequent development of anaerobic biofilms.

A comparison of the changes in the composition of the bacterial content of the periodontal pocket revealed a tendency toward a decrease in the number of resident coccus flora and *Lactobacilli*, accompanied by a gradual increase in anaerobic bacilliform flora (Figures 2 and 3).

The highest concentrations of actinomycetes were observed in patients with moderate periodontal tissue destruction. The isolation frequency of *Candida* fungi exhibited a positive correlation with the severity of periodontal lesions. Furthermore, the presence of dysbiosis led to an elevated frequency of detection for atypical *Enterobacteriaceae*.

Discussion.

Inflammatory periodontal diseases are among the most prevalent etiologies that prompt patients to seek consultation with a dentist. The significant prevalence of periodontal diseases underscores the importance of investigating its etiology and treatment modalities [13-15]. The primary etiological agent contributing to periodontal disease progression is the presence of periodontopathic microflora. Inflammatory phenomena in the gingival tissues, which gradually develop, result in significant destruction of the tissues surrounding the tooth and, ultimately, tooth loss. This decline in quality of life is a significant concern for patients and healthcare providers.

Dysbacteriosis, or microbial imbalance, plays a critical role in the progression of chronic periodontitis. As periodontitis advances, beneficial symbiotic bacteria are displaced by pathogenic anaerobes, particularly members of the red complex such as *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*. This dysbiotic shift results in impaired host-microbe homeostasis, sustained inflammation, and increased tissue destruction. The findings of the present study support this model, with more severe cases exhibiting a dominance of virulent species.

Prolonged chronic inflammation of the periodontal structures can affect the systemic state of the organism and contribute to activation of pathological processes in other systems and organs, as well as aggravation of already existing general systemic pathologies. The pathogenic potential of oral microflora is activated by the accumulation of dental plaque and the subsequent formation of stable plaque in proximity to periodontal tissues. All patients diagnosed with periodontal disease exhibit qualitative and quantitative alterations in microbial composition.

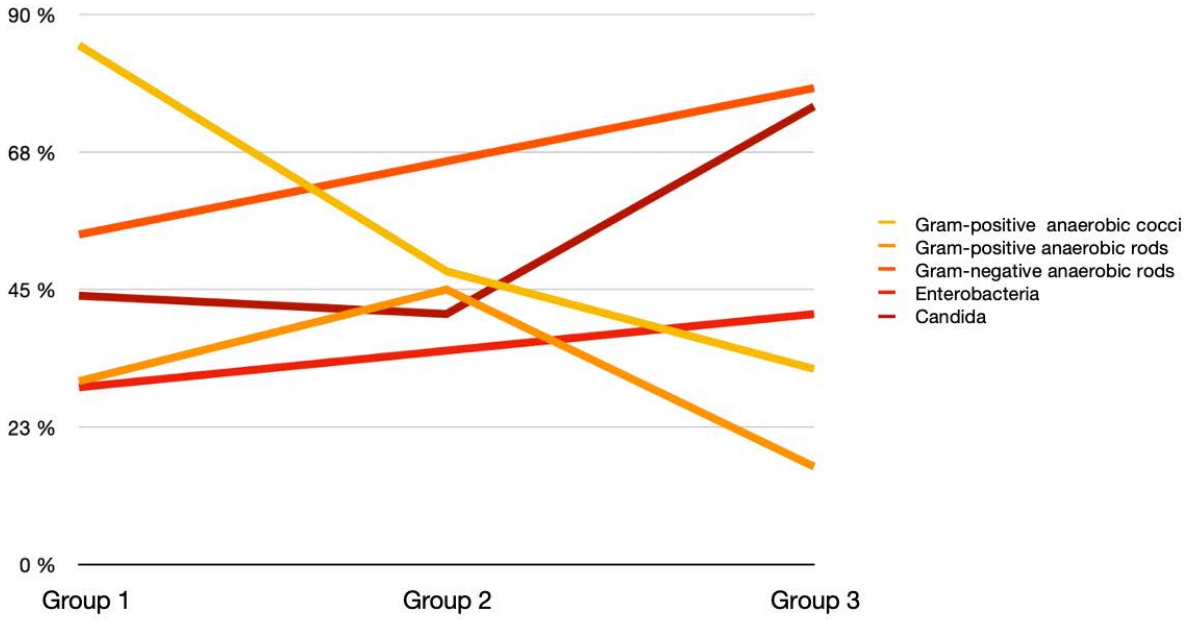

Figure 2. *Composition of periodontal pocket microflora in patients with different degrees of periodontal tissue damage.*

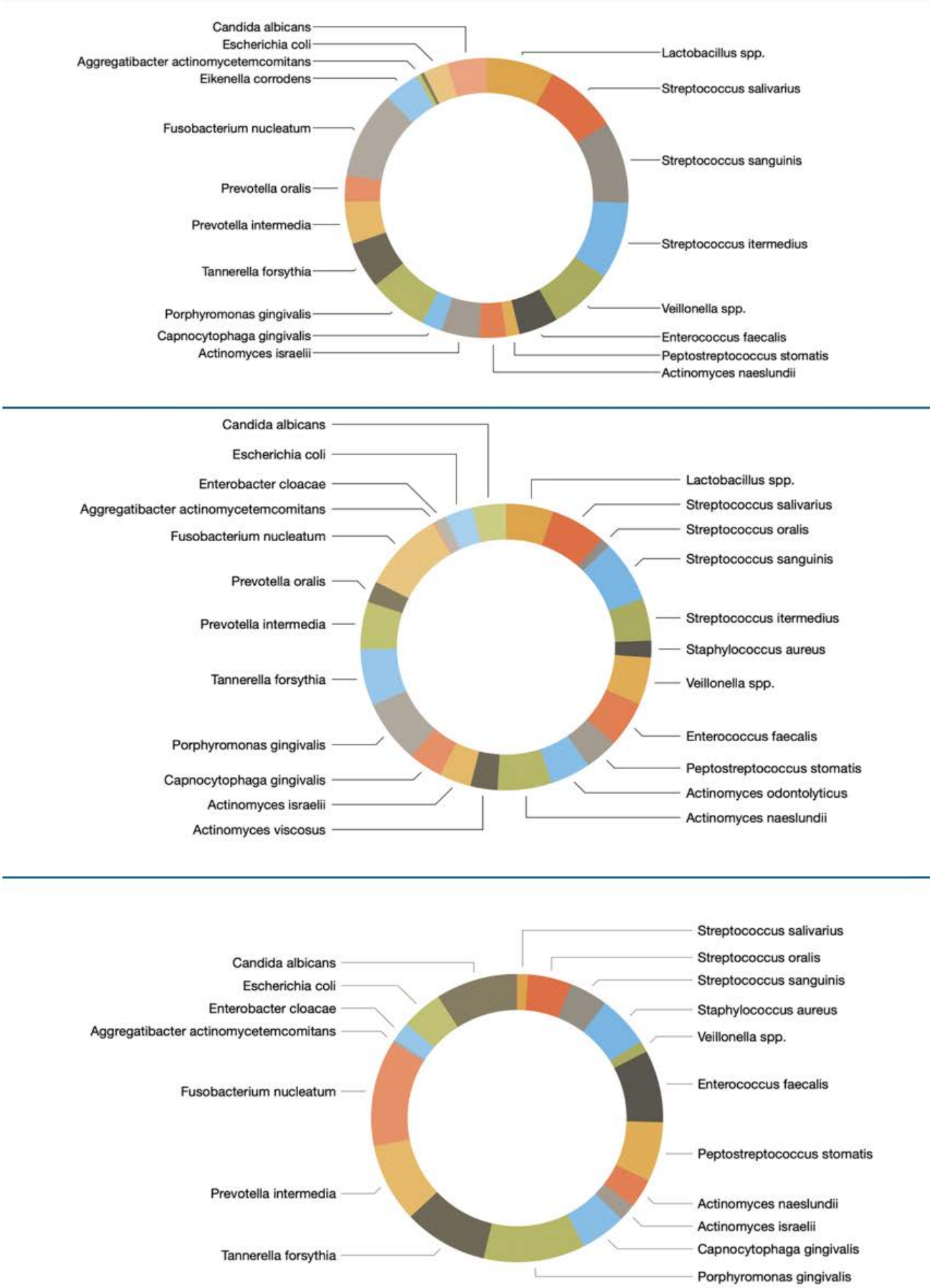

Figure 3. *Representative microbial profiles observed in patients with (A) mild, (B) moderate, and (C) severe chronic periodontitis.*

The composition of the microflora within the periodontal pockets is subject to variation, contingent upon the extent of bone tissue destruction. This destruction is accompanied by a decline in oxygen concentration, a phenomenon that concomitantly occurs with the progression of bone tissue degradation and the deepening of the periodontal pockets. Concurrently, there has been an observed shift towards an increase in the population of anaerobic microorganisms [16-18]. The aggregated data obtained in the present study demonstrated a decrease in the number of Gram-positive cocci and Gram-positive bacilli with increasing severity of periodontitis. Concurrently, there was an increase in the number and frequency of detection of Gram-negative bacilli, Enterobacteriaceae, and fungi. Consequently, there is a displacement of resident flora and predominance of opportunistic and pathogenic microorganisms.

The presence of periodontopathogenic bacteria (*Porphyromonas gingivalis, Tannerella forsythia*, and *Prevotella intermedia*) was detected in all patients, thereby confirming their direct involvement in the development of inflammatory periodontal diseases. This finding is in accordance with previously published literature data [15]. The presence of *Porphyromonas gingivalis* was detected in 79% of the samples, *Tannerella forsythia* in 67%, and *Prevotella intermedia* in 69%. These findings suggest a correlation between these species and periodontal diseases.

Among anaerobic microorganisms, *Aggregatibacter actinomycetemcomitans* plays an important role in the development of periodontitis [19]. Concurrently, the presence of this type of bacteria is associated with increased aggressiveness of the course of periodontitis and its progression [20]. In the present study, the prevalence of the specified outcome was observed to be 5%, 2%, and 3% in the three groups, respectively. A statistically significant correlation was not identified between the content of *Aggregatibacter actinomycetemcomitans* and the severity of periodontitis.

Current research also considers *Fusobacterium nucleatum* to be one of the major periodontopathogens. The bacterium's unique properties are attributable to the bilateral manifestation of its interaction with the macroorganism. *F. nucleatum* exhibits a dual nature of interaction with the host organism. In conditions of a healthy microbiome, this species is able to form symbiotic relationships [21]. However, under certain circumstances, it shows itself as a pathogenic agent that disrupts the microbiological balance and provokes the development of serious diseases of the oral cavity through interaction with other pathogenic microorganisms [22]. In practically all samples of our study, *F. nucleatum* was found. In addition, no statistically significant decrease in the concentration or frequency of isolation of *F. nucleatum* was observed among the three groups. This finding indicates that *F. nucleatum* is directly implicated in the development and progression of the condition under investigation.

The findings of the study indicated a consistent decline in the prevalence of beneficial bacteria, such as *Streptococci* and *Lactobacilli*, concomitant with an escalating incidence of invasive species, including *Enterobacter cloacae* and *Escherichia coli*. This phenomenon signifies the onset of dysbacteriosis and is in alignment with the observations reported by Denefil O. et al. [15].

In consideration of the extant data concerning the capacity of *Lactobacillus bacteria* to compete and the suppression of the growth of periodontopathogenic flora, it can be posited that there is a relationship between the displacement of *Lactobacillus* from the microbiome of the periodontal pocket and the progression of the disease [18]. Furthermore, a pronounced predominance of anaerobic flora is observed, which is known to exert a deleterious effect on periodontal tissues due to the secretion of various endotoxins.

A multitude of studies have evaluated the role of the fungus *Candida* in the development of periodontal diseases. These studies have demonstrated that the manifestation of commensal or pathogenic properties is contingent upon the localization and species composition of microbial communities [23]. The synergistic interaction of *Candida, F. nucleatum*, and *Porphyromonas gingivalis* has also been reported, underscoring the necessity for further study of this aspect [24]. The present study demonstrates a high detection rate of *Candida, F. nucleatum* and *Porphyromonas gingivalis*.

In addition to bacterial pathogens, fungi such as *Candida albicans* may influence the clinical course of periodontitis. *Candida* can adhere to periodontal tissues and form mixed biofilms with bacteria such as *Fusobacterium nucleatum* and *P. gingivalis*, enhancing their virulence. These synergistic interactions can exacerbate epithelial barrier damage and stimulate proinflammatory cytokine release. The presence of *Candida* in advanced periodontitis, as observed in this study, may thus represent a marker of microbial complexity and host immune dysfunction [25-28].

Conclusion.

The results of this study demonstrate that the qualitative and quantitative composition of periodontal microbiota changes significantly with disease severity. These findings may contribute to more precise microbial diagnostics and the development of targeted antimicrobial therapies. Understanding microbial shifts in dysbiosis can aid in early intervention and personalized prevention strategies for chronic periodontitis.

The microbial composition of the periodontal pockets content is contingent upon the degree of damage to periodontal structures. *Fusobacterium nucleatum* has been identified as a significant factor in the development of inflammatory diseases of periodontal tissues, with its presence documented in 99.4% of the samples examined.

As the severity of periodontitis progresses, a shift in the composition of microflora towards a predominance of gram-negative anaerobes becomes evident. The most prevalent phylotypes include *Bacteroidota* (*Porphyromonas gingivalis* (79%)), *Tannerella forsythia* (67%), and *Prevotella intermedia* (69%). As the severity of periodontitis escalated, there was a concomitant increase in the number and frequency of phylotype representatives.

A statistically significant correlation was not identified between the content of *Aggregatibacter actinomycetemcomitans* and the severity of periodontitis.

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