Analysis of microbiocenosis of a gingival sulcus and periodontal pockets of patients with periodontal diseases associated with systemic pathology

Olha Denefil[1] , Sofiia Chorniy[2] , Svitlana Boitsaniuk[3] , Nadiia Manashchuk[3] , Natalia Chornij[3] ,
Mariana Levkiv[3]* , Natalia Tverdokhlib[4] , Khrystyna Loza[4]

[1]Department of Pathophysiology, I. Horbachevsky Ternopil National Medical University, 46000 Ternopil, Ukraine
[2]Faculty of Medicine, I. Horbachevsky Ternopil National Medical University, 46000 Ternopil, Ukraine
[3]Department of Dental Therapy, I. Horbachevsky Ternopil National Medical University, 46000 Ternopil, Ukraine
[4]Department of Dental Surgery, I. Horbachevsky Ternopil National Medical University, 46000 Ternopil, Ukraine

*Correspondence: Mariana Levkiv, Department of Dental Therapy, I. Horbachevsky Ternopil National Medical University,
46000 Ternopil, Ukraine. levkiv@tdmu.edu.ua

Abstract

Aim: The aim is to analyze the microbiome of gingival sulcus and periodontal pockets of patients with
periodontal disease associated with systemic diseases.

Methods: A microbiological study was conducted to analyze the microflora of the periodontal pockets in
patients with different systemic pathologies and periodontal diseases. Plaque samples were collected from
the gingival sulcus and periodontal pockets, and they were subsequently cultured on nutrient medium and
glass plates.

Results: The microbiota of the gingival sulcus and periodontal pockets in patients with associated systemic
diseases in combination with periodontal disease was studied. The frequency of detecting the qualitative
composition of the microbiota in the periodontal niche of patients with periodontal diseases and systemic
diseases was determined. The research paper outlined groups of microorganisms isolated from periodontal
pockets of patients with periodontal and systemic diseases.

Conclusions: The degree of colonization by microorganisms differed slightly, while the frequency of
detection of specific populations of opportunistic bacteria increased in chronic generalized periodontitis
compared to chronic catarrhal gingivitis.

Keywords

Bacteria, microbiota, periodontal disease, periodontal pockets, systemic diseases

Introduction

Diseases of periodontal tissues constitute a significant problem in modern society due to their widespread and progressive course as they accompany tooth loss and compromised function of the maxillofacial system, decreasing patients' life quality [1, 2]. The main etiological and pathogenic factors of periodontal diseases are the aggressive microbes and their by-products [3, 4]. Periodontopathogenic oral microflora is the initial mechanism of the development of chronic inflammation in periodontal tissues. The hyperproductive growth of pathogenic microorganisms characterizes the dysbiosis of different endo-ecological niches of the oral cavity, leading to and supporting the severity and course of inflammatory-destructive processes in the periodontium. In the pathogenesis of inflammatory periodontal diseases, there is an interaction of two pathogenetic mechanisms: the influence of anaerobic microflora and the immunological reactivity of the human body. Periodontopathogenic species of bacteria differ from others in being highly adhesive, invasive, and toxic concerning periodontal tissues. Periodontopathogenic microorganisms have a wide range of pathogenic factors, which allows them to induce a prolonged inflammatory process. Bacteria can produce enzymes that inactivate antibiotics, which complicates treatment.

The researchers recognized that the course of the pathological process in the periodontium depends not only on the virulence of microorganisms in the oral cavity but also on the body's immune resistance [5]. Therefore, the state of the immune system and the resistance of periodontal tissues to bacterial invasion are crucial in the modern concept of the etiology and pathogenesis of periodontal diseases [6]. Herpesvirus infections may impede the antibacterial host defense and alter periodontal cells to predispose bacterial adherence and invasion [7]. One of the problems of modern clinical dentistry is the increase in the incidence of chronic inflammatory pathology of the periodontium, which develops because of secondary immune deficiency [8].

General reasons that determine the resistance of periodontal tissues to pathogenic influences are also important [9]. The relationship between systemic and periodontal pathology is apparent. Systemic pathology is a risk indicator or risk factor in periodontal diseases [10, 11].

Systemic disorders can impact various systems in the body, including the circulatory, respiratory, digestive, immune, and endocrine systems. They often involve complex interactions between different organs and tissues. Many systemic disorders are chronic, persisting over time, and may require ongoing management or treatment. Some systemic conditions have identifiable risk factors, such as genetics, lifestyle factors, environmental exposures, and infections. Diagnosing systemic diseases involves a combination of medical history, physical examination, laboratory tests, and sometimes specialized procedures.

The endocrine system is one of the most significant internal factors affecting the immune response. Multifunctional relationships between the gastrointestinal, immune, and endocrine systems are extremely important in the etiology and pathogenesis of periodontal disease [12]. In the structure of endocrine pathology, one of the leading roles belongs to thyroid gland disorders, especially primary hypothyroidism. Hypofunction of the thyroid gland affects the functional state of many organs and systems, particularly, the tissues of the dento-alveolar complex, the metabolism of bone tissue, and the state of the immune system. Destruction of immunological parameters and the development of a secondary immunodeficiency in the course of hypothyroidism were established [13]. However, the connection between the changes in the oral cavity microbiota due to systemic diseases and the development of periodontal tissue diseases has not been sufficiently studied yet [14].

Many taxonomic refinements have developed as split-offs from previously established genera and species. Classical culture and molecular methods have advantages and drawbacks. It makes sense to recognize their respective attributes to gain the most from studies on periodontal microflora. A few techniques, such as Bergey's method, 16S ribosomal RNA (rRNA) sequencing, and next generation sequencing (NGS), can be used to identify bacterial taxa.

Bergey's manual method

Bergey's manual of systematic bacteriology [15] was used as the early standard for identification. It is the leading resource for determining the identity of prokaryotic organisms, emphasizing bacterial species using every characterizing aspect. The advantages of this method are the assessment of living microorganisms, the ability to recognize viable cells in a sample, the easiness of quantitating cells in a piece, and its high sensitivity with appropriate media [16].

16S rRNA sequencing

The use of molecular techniques for identifying bacteria in periodontology is relatively recent. 16S rRNA sequencing is a molecular biology technique targeting the 16S rRNA gene. This gene is present in all bacteria and contains variable regions that can be used for taxonomic classification. It provides high taxonomic resolution at the genus and species levels, making it suitable for identifying and characterizing individual microbial species within a sample. 16S rRNA sequencing involves PCR amplification of the 16S rRNA gene region, followed by DNA sequencing of the amplicons. The obtained sequences are compared to reference databases to assign taxonomic classifications to the microbes present in a sample.

NGS

NGS is a high-throughput sequencing technology that has revolutionized the field of genomics and metagenomics. It enables the rapid and cost-effective sequencing of large volumes of DNA or RNA. This method can be applied to various types of sequencing, including whole-genome sequencing, metagenomic sequencing, transcriptome analysis, and more. It is not limited to 16S rRNA genes and can sequence entire genomes or other genetic elements. NGS generates vast amounts of sequencing data, providing a comprehensive view of the genetic information present in a sample. It allows for a deeper understanding of community microbial diversity and functional potential within a community. NGS can provide both taxonomic information (similar to 16S rRNA sequencing) and functional information by sequencing the entire genetic content of organisms in a sample.

Materials and methods

The microbiocenosis of the gingival sulcus and periodontal pockets was investigated to achieve the research goals. In total, 539 patients were examined in the main group. Among them, 83 patients with primary hypothyroidism, 62 patients with chronic colitis, 102 patients with chronic pancreatitis, 112 patients with peptic ulcer disease of the stomach and duodenum, 60 patients with gastroduodenal pathology associated with *Helicobacter pylori*, and 120 patients with combined pathology of various parts of the gastrointestinal tract and periodontal diseases. Such inclusion and exclusion criteria for patients in a survey were employed, as shown in Table 1.

Table 1. Inclusion and exclusion criteria of patients into a survey

Inclusion criteria	Exclusion criteria
A compensated form of a systemic disease, no more than ten years.	Decompensated form of a systemic disease, more than ten years in combination with another systemic illness.
Patients treated with a pharmacotherapeutic method.	Surgical interventions as a method of treatment of a systemic disease.
The age of the patient is from 18 years to 60 years.	The patient's age is over 60 years.
Patients with systemic pathology and the presence of teeth.	Patients with systemic pathology and absence of teeth.

Patients included in the study were under dispensary observation and outpatient treatment in the endocrinology department of "Ternopil University Hospital" and the polyclinic department of "City Communal Hospital No. 3". Dentists examined them on an outpatient basis at the Department of Dental Therapy of "I. Horbachevsky Ternopil National Medical University". Department doctors verified the

leading systemic diseases based on valid national and international protocols (according to clinical and instrumental examination data and international classification of diseases).

The control group comprised 30 patients with clinically healthy periodontium without accompanying systemic pathology. In diagnosing periodontal diseases, basic periodontal diagnostic parameters were used: probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level. Only patients aged 18–60 were included in the studied groups (main, control) to exclude age-related pathology.

A criterion for the diagnosis of chronic generalized periodontitis

The absence of a dentogingival junction was the principal criterion for diagnosing chronic generalized periodontitis (CGP). The stage of severity was determined based on the depth of periodontal pockets and the degree of destruction of bone tissue. Thus, a mild degree of CGP is characterized by periodontal pockets less than 3 mm deep and the X-ray pattern confirming signs of initial destruction of the inter-dental septa. The depth of periodontal pockets varied from 3 mm to 6 mm in patients with a moderate stage of CGP; I–II degree of pathologic tooth mobility (according to tooth mobility index by Miller scale in Fleszar modification) was frequently revealed during the examination. According to the data obtained by X-ray examination, the destruction of the interdental septa's cortical plate and bone tissue was equal to 1/2–1/3 of the length of the tooth root.

Periodontal pockets more than 6 mm deep, II–III degree pathological tooth mobility, cortical plate, and bone tissue destruction by more than 1/3 of the tooth root length are typical for the severe stage of CGP. Cortical plate and bone tissue destruction was revealed by X-ray examination.

Smears from periodontal pockets to study the quantitative and qualitative composition of the microflora of this biotope were taken. Our team collected plaque samples with a sterile inoculation needle from periodontal pockets and immediately applied them to nutrient medium and glass plates.

Researchers took samples from the patient's gingival sulcus in the morning hours on an empty patient's stomach with a sterile standard ISO (International Organization for Standardization) size 30 paper endodontic pin (DiaDent), 1 cm long, which, after soaking, the tooth was placed in a sterile saline solution and washed thoroughly.

Sugar meat-peptone agar and blood agar were used to isolate aerobic, facultatively anaerobic, and microaerophilic microorganisms [growth mediums (dry), Farmaktiv LLC]. The identification of isolated pure cultures was carried out according to morphological, cultural, and biochemical characteristics following generally accepted methods and the identifier of bacteria by Bergey (1994) [15]. Bergey's manual of systematic bacteriology is the primary resource for determining the identity of prokaryotic organisms, emphasizing bacterial species, using every characterizing aspect. An isolated pure culture was identified by a complex of morphological, cultural, and biochemical properties (sets MLT00014 STREPTOtest 16_P, MLT00012 STAPHYtest 16_R, Erba Lachema).

The presence of *Fusobacterium, Veillonella*, *Prevotella*, and *Porphyromonas* was determined according to morphological and cultural properties. Smears were taken from periodontal pocket content and stained using Gram and Romanowsky-Giemsa methods, followed by immersion microscopy. To inoculate bacteria, the method of sectoring on dense nutrient media was employed to determine the specific content of each group of microorganisms in 1 g of biomaterial. The number of colony-forming units (CFUs) expressed the degree of microbial colonization. After counting the CFU, the data was converted into decimal logarithms (lg CFU).

The Bioethics Committee of I. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine approved the study protocols (excerpt from protocol No. 73) on April 3, 2023. All the patient investigations conformed to the principles outlined in the Council of Europe Convention on Human Rights and Biomedicine (April 4, 1997), the World Health Association Helsinki Declaration on Ethical Principles for Scientific Research with Human Participation (1964–2000), and the Order of the Ministry of Health of Ukraine No. 281 of November 1, 2000; Declaration of Helsinki "World Medical Association Declaration of

Helsinki Ethical Principles for Medical Research Involving Human Subjects" (2001), Code of Scientist of Ukraine (2009). Doctors informed every patient about the study's purposes. Patients signed their "consent of the patient".

Statistical analysis and testing of hypotheses for the resulting findings were performed by means of parametric criterion Z-test [17, 18].

Results

The researchers observed significant qualitative and quantitative changes in the character of microbiocenosis of periodontal pockets and gingival sulcus in patients with a concomitant gastrointestinal tract pathology compared to similar, practically healthy patients.

Patients with thyroid gland dysfunction

Microbiological studies have shown that various microorganisms inhabit the gingival sulcus and periodontal pockets in chronic catarrhal gingivitis and CGP in patients with primary hypothyroidism. In patients with hypothyroidism, 536 strains of microorganisms that were either aerobic, facultative aerobic, or anaerobic were identified.

The degree of general insemination of the studied microbial oral ecosystem of patients with primary hypothyroidism and periodontal disease was on average 4.4 lg CFU/mL ± 1.2 lg CFU/mL, ranging from 2.7 lg CFU/g ± 0.2 lg CFU/g (non-pathogenic *Neisseria*) to 6.8 lg CFU/g ± 0.2 lg CFU/g (oral streptococci, Table 2). In this study, the sample mean is denoted as M and the standard error as m.

Table 2. Microbiome of periodontal pockets in patients with primary hypothyroidism in combination with periodontal disease

Microorganism		Main group		Control group	
		n = 83		n = 30	
		Frequency of detection (%)	Colonization density (lg CFU/mL)	Frequency of detection (%)	Colonization density (lg CFU/mL)
			(M ± m)		(M ± m)
Facultative anaerobic bacteria					
Gram-positive cocci	α-hemolytic streptococci	93.30	6.80 ± 0.20	100.00	6.75 ± 0.11
	β-hemolytic streptococci	51.70	5.20 ± 0.20	26.70	3.39 ± 0.17**
	Coagulase-positive staphylococci	75.50	5.60 ± 0.10	33.30	3.94 ± 0.16**
	Coagulase-negative staphylococci	78.30	4.40 ± 0.19	46.70	4.02 ± 0.18**
	Corynebacterium	33.30	3.60 ± 0.10	13.30	1.44 ± 0.17**
Gram-negative rods	*Escherichia coli*	35.00	2.30 ± 0.10	10.00	0.13 ± 0.08**
	Klebsiella	15.00	1.22 ± 0.30	0.00	-
Non-pathogenic *Neisseria*		55.00	2.73 ± 0.21	43.30	5.32 ± 0.11**
Microaerophilic bacteria					
Lactobacilli		68.30	3.41 ± 0.21	53.30	5.77 ± 0.18**
Obligate anaerobic bacteria					
Fusobacteria*		68.30	-	100.00	-
*Veillonella**		66.70	-	100.00	-
Prevotella		43.30	-	0.00	-
Porphyromonas		83.30	-	0.00	-
Spiral shaped bacteria					
*Spirochaeta**		75.00	-	100.00	-
Saccharomycetaceae fungus					
Candida fungi		51.70	3.20 ± 0.10	6.70	1.23 ± 0.21**

*: microorganisms that are identified by the microscopic method; **: $P < 0.05$; -: not detected

Microorganisms' colonization density of periodontal pockets has reached clinically significant indicators. Half of the patients have in periodontal pockets potentially pathogenic Gram-negative bacteria belonging to the following genera: *Veillonella* (in 66.7%), *Fusobacterium* (68.3%), *Spirochaeta* (75.0%); pathogenic β-hemolytic streptococci (51.7%); non-pathogenic *Neisseria* (55.0%). Gram-negative Enterobacteriaceae were in some patients, in particular strains of *Escherichia coli* and *Klebsiella* (35.0% and 15.0%, respectively). The density of their colonization of periodontal pockets was 2.3 lg CFU/mL ± 0.1 lg CFU/mL and 1.2 lg CFU/mL ± 0.3 lg CFU/mL, respectively.

The studies showed that the composition of the microflora of periodontal pockets in patients with thyroid hypofunction was poly-microbial and represented by various associations of microorganisms. Most of the associations consisted of 5 or more species of bacteria (in 78.3% of patients). In 15% of cases, associations with 3–5 types of microorganisms, only four patients (6.7%) have two-component associations.

Microbiomes of periodontal pockets are prevalent in species of anaerobic and facultative anaerobic microorganisms. Most patients with hypothyroidism have the leading "biomarker bacteria" and periodontopathogenic microorganisms: *Prevotella*, *Porphyromonas*, and *Spirochaeta*.

An α-hemolytic streptococci was isolated in almost all patients (93.3%). The density of their colonization of the periodontal ecological niche in average of 6.8 lg CFU/mL ± 0.2 lg CFU/mL is less than standard data. Only 68.3% of patients have lactobacilli cultures; their density was equal to 3.4 lg CFU/mL ± 0.2 lg CFU/mL.

It indicates the influence of systemic disease on the development of dysbiotic processes in the oral cavity. *Porphyromonas* strains, which belong to Gram-negative non-spore-forming anaerobes, were found in most patients with hypothyroidism (83.3% of patients) and coagulase-positive and coagulase-negative staphylococci in 75.0% and 78.3% of cases, respectively.

Patients with gastroduodenal pathology

Analysis of the results of microbiological studies of patients with gastroduodenal pathology associated with *Helicobacter pylori* showed that the microflora of the pockets was poly-associative. Potentially pathogenic and pathogenic microorganisms were found in all patients. This microflora represents associations of various species of the *Streptococcus* genus and *Staphylococcus* genus, as well as lactic acid bacteria, fungi, and anaerobic microorganisms. On average, associations containing 3 to 6 types of microorganisms were inoculated from the sample. Patients with gastroduodenal pathology have aerobic-anaerobic associations in 40% of cases, aerobic-anaerobic-fungal in 48%, and anaerobic-fungal associations in 12% of observations. *Streptococcus* spp. represented the main part of the aerobic microflora. The dominant species was *Staphylococcus haemolyticus* (77.3%). Analysis of the species composition of the anaerobic microflora of the periodontal pockets showed significant inoculation with Gram-negative microorganisms (*Bacteroides forsitus*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*) (Figure 1). *Peptostreptococcus anaerobius* was detected in 35% cases in the whole spectrum of Gram-positive anaerobic microorganisms. The analysis of the quantitative composition of the microflora of the periodontal pockets showed that contamination by the previously mentioned types of bacteria in the main group averaged from 1×10^5 to 1×10^8 CFU/mL. Microscopic studies revealed significant microbial contamination of periodontal pockets of patients with gastric and duodenal ulcers. There was a shift in the microbial spectrum toward an increase in Gram-negative rods, associated with a decrease in Gram-positive rods and Gram-negative cocci. During bacteriological studies, staphylococci and streptococci were most often identified in diagnostically significant concentrations among facultative anaerobic microorganisms and among obligate anaerobes—*Fusobacterium* spp., *Peptostreptococcus* spp., *Prevotella* spp., *Porphyromonas gingivalis*. In high concentrations from 3×10^5 CFU/mL to 2×10^8 CFU/mL, *Fusobacterium* was isolated in 77.2% of patients. The dominance of these microorganisms in the microbial associations of periodontal pockets is characteristic. A high frequency of detection (67%) of *Candida* fungi with a colonization rate of 1×10^2–1×10^4 CFU/mL was determined.
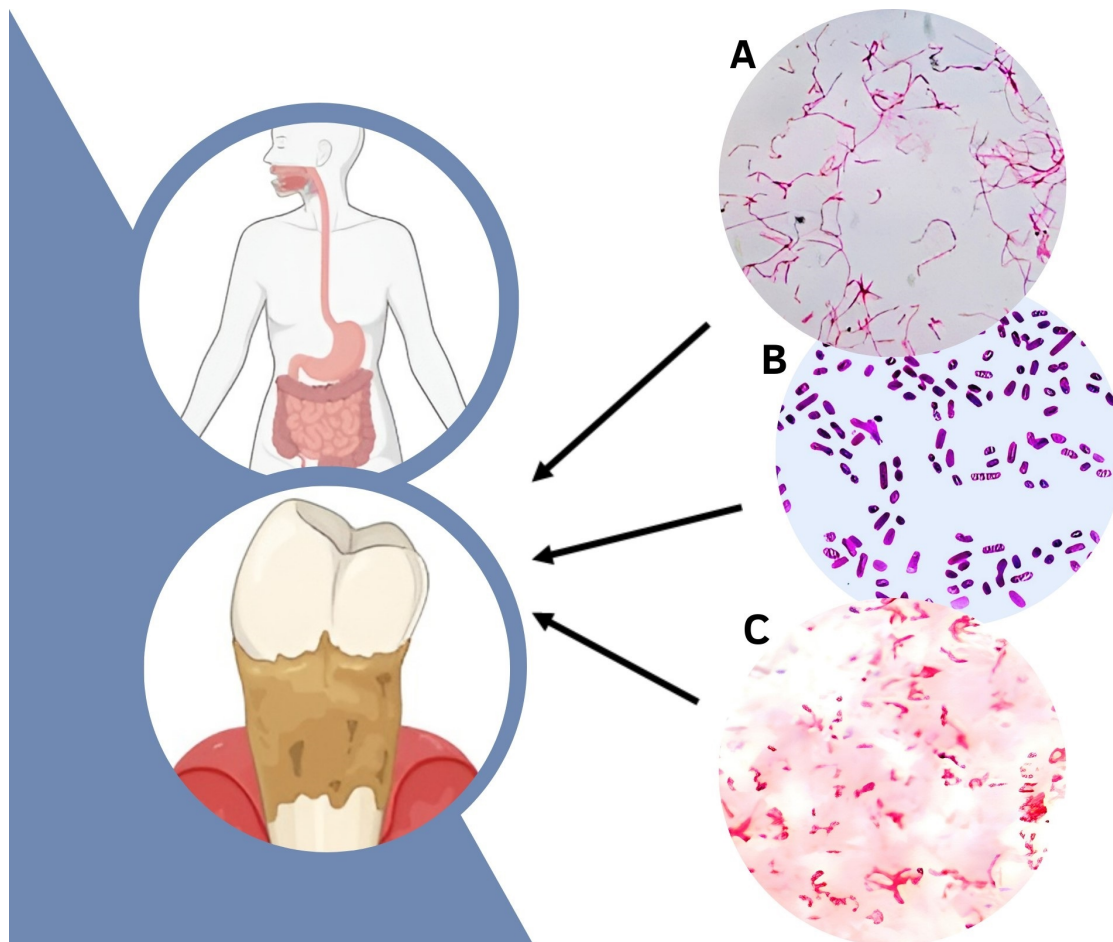
Figure 1. Microorganisms that prevail in patients with periodontal tissue pathology and gastroduodenal pathology. (A) *Fusobacterium nucleatum* (light microscopy, 1000× magnification, Gram stain); (B) *Prevotella intermedia* (light microscopy, 1000× magnification, Gram stain); (C) *Porphyromonas gingivalis* (light microscopy, 1000× magnification, Gram stain). The authors created the figure on the platform Canva (https://www.canva.com/) and with BioRender.com (https://app.biorender.com). Subpictures are original

Patients with chronic colitis

The microflora of periodontal pockets of patients with colitis was dominated by cocci, which accounted for 49.8% of all cultured microorganisms. Gram-positive facultative anaerobic cocci belonging to the genera *Staphylococcus* and *Streptococcus* were dominant (Figure 2). In addition, the spectrum of microbiocenosis included anaerobic gram-negative rods, which made up 35.0% of the microbial community, *Candida* fungi (11.0%), and *Spirochaeta* (7.0%). The number of lactobacilli decreased (6.0%), which indicates dysbiosis of the periodontal pockets. Enterobacterium, such as *Escherichia coli* and *Klebsiella* spp., occupied the smallest segment in the microbiocenosis of the periodontal pockets (2.7%), which belonged to invasive microflora.

The degree of total microbial colonization of the gingival sulcus of patients with catarrhal gingivitis was 5.62 lg CFU/mL ± 2.32 lg CFU/mL. The periodontal pocket microorganism colonization density was 6.17 lg CFU/mL ± 2.56 lg CFU/mL, which is a few times higher than in patients with chronic gingivitis (Figure 3).

Patients with chronic pancreatitis

The microbiological studies of samples from patients with chronic pancreatitis showed that various microorganisms inhabit periodontal pockets and gingival sulcus (Table 3). The degree of its total colonization ranged between 7.23 lg CFU/mL ± 0.09 lg CFU/mL in the case of chronic catarrhal gingivitis and 7.37 lg CFU/mL ± 0.15 lg CFU/mL in the case of CGP. Microorganisms in periodontal pathology were inoculated from gingival and periodontal pockets with approximately the same inoculation density. Most microorganisms were isolated within the same order, except for *Escherichia coli*. Their number was a few times higher in patients with CGP than in those with chronic catarrhal gingivitis (2.19 lg CFU/mL ± 0.13 lg CFU/mL *vs.* 1.83 lg CFU/mL ± 0.10 lg CFU/mL, respectively). α-hemolytic streptococci, *Spirochaeta*,
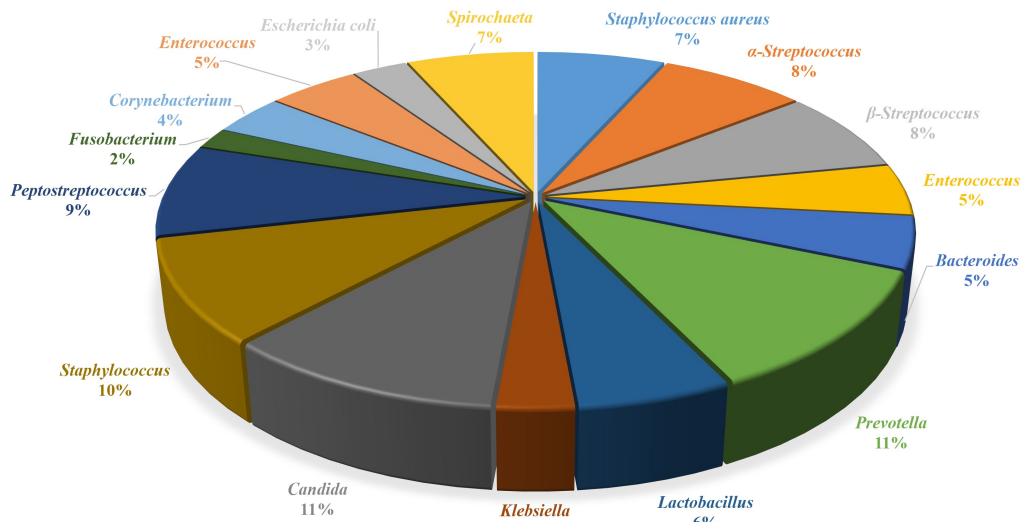
Figure 2. Qualitative composition of the microbiocenosis of the periodontal pocket of patients with chronic colitis
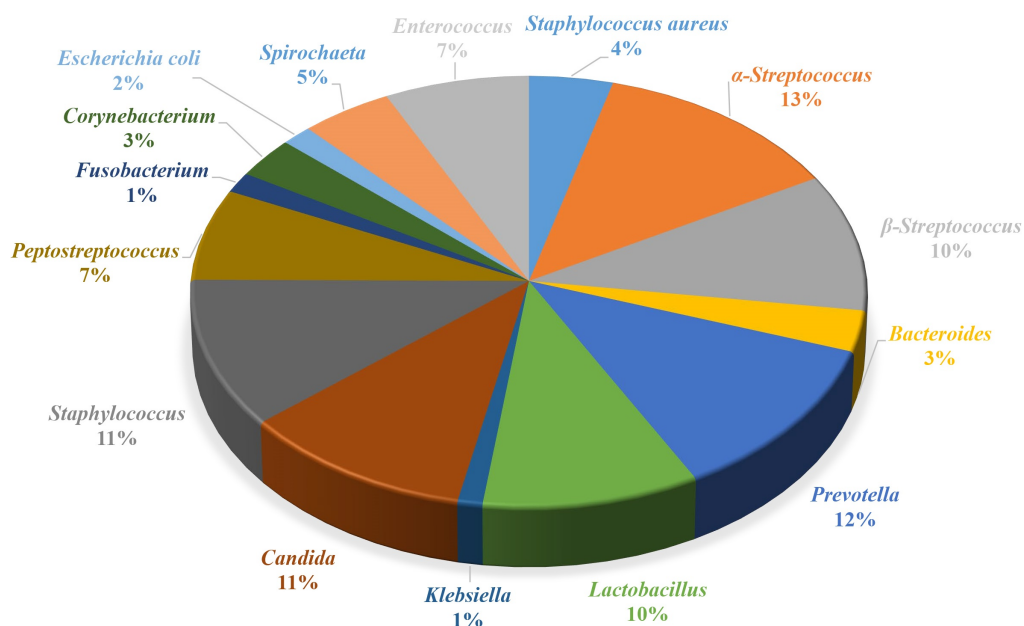

Figure 3. Qualitative composition of the microbiocenosis of the gingival sulcus of patients with chronic colitis

Fusobacterium, and *Veillonella*, which formed the main normal microflora of gingival and periodontal pockets, dominated this biotope. Staphylococci (78.6%) and lactobacilli (73.8%) were patients' second most frequently detected bacteria. In patients with CGP, the frequency of detection of coagulase-positive staphylococci was approximately the same (77.8%), while the frequency of coagulase-negative staphylococci was slightly lower (66.7%). Lactobacilli, which also belong to the normal microflora of the oral cavity and gum pockets, were cultured in periodontitis with a frequency almost 20% lower than in gingivitis. Saprophytic *Neisseria* and *Corynebacterium* were also detected less often in the second case. The frequency of detection of β-hemolytic streptococci, which, like staphylococci, are considered pyogenic cocci and play a significant role in the development of the inflammatory process, has increased significantly (from 26.2% in chronic catarrhal gingivitis to 72.2 % in CGP). At the same time, *Escherichia coli*, *Klebsiella*, and *Candida* fungi were cultured more frequently from the contents of gingival sulcus and periodontal pockets. Microbiological studies of the microbiocenosis of periodontal pockets of patients with CGP and gastrointestinal tract pathology revealed that the frequency of anaerobic microorganisms' detection was higher than in patients with generalized periodontitis without concomitant systemic pathology, i.e., *Helicobacter pylori* (7.0 times), *Bacteroides forsythias* (4.2 times), *Fusobacterium nucleatum* (5.2 times), *Prevotella intermedia*, and *Porphyromonas gingivalis* (5.0 times).

Table 3. Colonization density and frequency of detection of microorganisms in the gingival sulcus and periodontal pockets

Microorganism	Main group n = 102		Control group n = 30	
	Frequency of detection (%)	Colonization density (lg CFU/mL) (M ± m)	Frequency of detection (%)	Colonization density (lg CFU/mL) (M ± m)
Coagulase-positive staphylococci	77.8	4.44 ± 0.11	33.3	3.94 ± 0.16**
Coagulase-negative staphylococci	66.7	4.50 ± 0.14	46.7	4.02 ± 0.18**
α-hemolytic streptococci	100.0	6.82 ± 0.11	100.0	6.75 ± 0.11**
β-hemolytic streptococci	40.0	5.39 ± 0.19	26.7	3.39 ± 0.17**
Neisseria	55.0	6.54 ± 0.16	43.3	5.32 ± 0.11**
Lactobacilli	73.8	3.95 ± 0.12	53.3	5.77 ± 0.18**
Klebsiella	15.0	1.80 ± 0.21	0.0	-
Escherichia coli	41.7	2.08 ± 0.11	10.0	0.13 ± 0.08**
Candida fungi	60.0	3.21 ± 0.11	6.7	1.23 ± 0.21**
Corynebacterium	33.3	3.49 ± 0.09	13.3	1.44 ± 0.17**
Spirochaeta*	100.0	-	100.0	-
Fusobacterium*	100.0	-	100.0	-
Veillonella*	100.0	-	100.0	-

*: microorganisms that are identified by the microscopic method; **: $P < 0.05$; -: not detected

Discussion

Systemic disorders can significantly influence a person's quality of life and may require long-term management and care. Research has shown a bidirectional relationship between CGP and certain systemic disorders. CGP is characterized by chronic inflammation in the gums. It is a kind of infectious disease initiated by subgingival periodontal pathogens, which destroy ligament and alveolar bone supporting the teeth. Ultimately, it results in the loss of the affected teeth, compromising patients' well-being. The systemic inflammatory response triggered by periodontal infection can contribute to developing or exacerbating other systemic inflammatory conditions, such as cardiovascular disease, diabetes, and rheumatoid arthritis. Oral bacteria from periodontal infections can enter the bloodstream and potentially spread to other body parts. It can lead to infections in distant organs and tissues.

However, maintaining good oral hygiene, seeking regular dental care, and managing risk factors (i.e., smoking, alcohol intake, drug intake, etc. [19]) can help reduce the risk of gum disease and associated systemic health problems. Additionally, individuals with systemic disorders should be aware of the potential impact of their condition on oral health and consult with healthcare professionals as needed.

It is vital to comprehend the histology of clinically healthy tissues and inflamed gingival/periodontal tissues to understand periodontal pathogenesis better. A gingival crevice is the place where the pathological inflammatory process in the periodontium occurs and then progresses. This low-grade inflammation occurs in response to the continued presence of bacteria and their by-products in the gingival sulcus. A continuous exudation of fluid from the gingival tissues enters the crevice and seeps out as gingival crevicular fluid [20]. Toxins, by-products of microorganisms and enzymes, are inactivated by cellular and plasma components of the blood, which, in response to the action of pathogenic stimuli, penetrate from the capillaries through the tissue base into the sulcus. Therefore, even in normal conditions, there is always a particular microbial attack and the reaction of protective complexes to it in the gingival crevice, which belongs to the inflammatory category [21]. An explicit confirmation of this is the inevitable presence in the gingival sulcus of at least a minimal amount of gingival fluid, which is blood exudate from the capillaries network, as well as single leukocytes, exfoliated epithelial cells, a set of enzymes and proteins [22].

In all patients, there is a violation of the qualitative and quantitative composition of the microflora of the ecological niche of the periodontium. Dysbiosis was detected in all patients, as evidenced by a decrease

in the frequency of culture of obligate oral microorganisms (i.e., streptococci, lactobacilli), an increase in the number of staphylococci, and the detection of invasive species of bacteria (i.e., *Klebsiella* and *Escherichia coli*).

Pathological disorders occur when the intensity of the microbial attack increases sharply. It happens in several cases: firstly, when the number of clusters of microorganisms increases; secondly, when, in addition to the usual saprophytic bacteria, pathogenic microorganisms appear in their composition [23]. As a rule, these are *Spirochaeta*, mobile forms of cocci, but they are mainly anaerobes. The peculiarity of these pathogenic bacteria is as follows: if saprophytic bacteria release exotoxins, to which tissue structures are sufficiently tolerant, then anaerobes release endotoxins, actively damaging cells, connective tissue formations, and the main substance. These bacteria appear with massive accumulations of microbial plaque, as they actively reproduce only in deep, oxygen-deprived layers. The reduction of specific and non-specific local and general protection mechanisms is usually accompanied by a sharp activation of constantly present microbial clusters. The consequence of this is the development of a clinically pronounced inflammatory reaction, the purpose of which is to neutralize tissue-destroying enzymes and toxins released by microbes, as well as to destroy the microbial cells.

Anaerobic microorganisms play the leading periodontopathogenic role among microorganisms [24, 25]: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, etc. It was established that the qualitative and quantitative composition of the microflora of the periodontal and gingival pockets is influenced by the presence of concomitant chronic pathology of the body's digestive system. It is impossible to unequivocally talk about which microorganisms are the main ones in the pathogenesis of periodontal diseases due to the variability of the microbial composition of dental plaque. When studying the relative ratio of microbes in the subgingival areas of healthy and affected periodontium, it was found that cocci, straight rods, filaments, and fusiform bacteria predominate in healthy areas. In sites with affected periodontium, the number of motile rods and spirochetes increases proportionally to the growth of the plaque index, periodontal index, and pocket depth. Recently, great importance has been given to the synergism of microbes in the pathogenesis of periodontal diseases [26, 27]. Bacterial antagonism also plays a significant role in microbial colonization of the oral cavity. For example, hemolytic *Streptococcus* produces a high-molecular peptide and *Streptococcus mitis*—a substance of a non-protein nature, which *in vitro* inhibits black-pigmented *Bacteroides*. On the other hand, haematin produced by *Bacteroides* nigra inhibits the growth of *Streptococcus mutans* and a protein-like compound from *Bacteroides* nigra (melanin) inhibits, *Bacteroides oralis*, *S. mutans*, *Streptococcus salivarius*, and some species of *Actinomyces*. The pathogenic potential of bacteria is determined by the direct harmful effect of their by-products and by triggering immune response mechanisms and inflammatory reactions [28, 29]. Many microorganisms found in periodontitis produce powerful necrotizing exotoxins and enzymes, such as collagenase, elastase, fibrinolysin, hyaluronidase, etc., which cause the destructive effect of periodontal tissues. *Bacteroides* release volatile sulfur compounds that increase the permeability of the mucous membrane of the oral cavity [30, 31]. Many *Bacteroides* can produce enzymes that inactivate antibiotics—β lactamase, cephalosporinase, and penicillinase. Thus, the occurrence, severity, and intensity of the development of inflammatory and destructive periodontal diseases directly depend on the qualitative and quantitative composition of the microflora of the oral cavity.

The microflora of periodontal pockets is diverse and depends on the nature of the disease [32]. There is no doubt that populations of microorganisms that colonize the oral cavity are involved in pathological processes. In this regard, studying the microflora of periodontal pockets is essential for assessing the quality of treatment strategies [33, 34].

Limitations and prospects of study

Study's limitations

This study's limitations imply that microorganisms are extracted from their environment and then studied microscopically or in culture. There is clear evidence that not all organisms in the periodontal pockets are

subsequently cultivated, as revealed by microscopy of direct smears and correlative light and electron microscopy. The diversity of microbial communities is usually based on complex environmental, nutritional, and communicative interactions between species. By necessity, taking a sample underestimates the number and variety of species present in the original ecological niche. The sampling method, transport conditions, storage, culture (dilution, handling, media, incubation, and atmosphere), and laboratory sub-culture all have the potential to bias the resulting types and numbers of species.

Prospects of research

Based on research results within the framework of evidence-based medicine, doctors can optimize treatment strategies and provide individual approaches in selecting pharmacotherapy for patients with combined pathologies. Moreover, treatment regimens will be developed for patients with periodontal diseases in the case of comorbidity.

Further research ideas

Cytokine study can facilitate a more thorough correlation analysis and will be a crucial contribution to current clinical research in the future. It is also suggested to use "omics" technology for future research, which allows massively parallel analysis of molecules, including genes, transcripts, and proteins. This technology can quickly generate a massive amount of data in a single experiment, so it features a unique high output.

Conclusions

According to the research results, we can assume that a significant prevalence of periodontal diseases and lesions of the hard tissues of teeth in patients with associated systemic pathology indicates the direct influence of these concomitant diseases on the state of the patient's dental health.

The degree of colonization by microorganisms in these pathologies differed slightly. At the same time, the frequency of detection of specific populations of opportunistic bacteria (i.e., β-hemolytic streptococci, *Escherichia coli*, *Klebsiella*, *Candida* fungi) increased in CGP compared to chronic catarrhal gingivitis.

The microbiological study of periodontal and gingival pockets of patients with periodontal pathology accompanied by systemic diseases indicates the need for a differentiated approach to antibacterial therapy.

It is important to emphasize that systemic diseases, disorders, or conditions do not cause periodontitis but instead predispose, accelerate, or otherwise increase disease progression.

Abbreviations

CFU: colony-forming unit

CGP: chronic generalized periodontitis

NGS: next generation sequencing

rRNA: ribosomal RNA

Declarations

Acknowledgments

Author contributions

OD: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. SC: Conceptualization, Investigation, Writing—review & editing. SB and KL: Investigation, Data curation,

Formal Analysis, Writing—review & editing. NM: Conceptualization, Investigation, Writing—original draft. NC and ML: Validation, Writing—review & editing, Supervision. NT: Investigation, Writing—original draft, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The Bioethics Committee of I. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine approved the study protocols (excerpt from protocol No. 73) on April 3, 2023. All the patient investigations conformed to the principles outlined in the Council of Europe Convention on Human Rights and Biomedicine (April 4, 1997), the World Health Association Helsinki Declaration on Ethical Principles for Scientific Research with Human Participation (1964–2000), and the Order of the Ministry of Health of Ukraine No. 281 of November 1, 2000; Declaration of Helsinki "World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects" (2001), Code of Scientist of Ukraine (2009). All patients' examinations were done with informed consent.

Consent to participate

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Not applicable.

Availability of data and materials

The datasets for this study can be found in the corresponding author upon reasonable request.

Funding

Not applicable.

Copyright

References

1. Bawadi HA, Khader YS, Haroun TF, Al-Omari M, Tayyem RF. The association between periodontal disease, physical activity and healthy diet among adults in Jordan. J Periodontal Res. 2011;46:74–81.
2. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J. 2013;7:1016–25.
3. Raja M, Ummer F, Dhivakar CP. Aggregatibacter actinomycetemcomitans – a tooth killer? J Clin Diagn Res. 2014;8:ZE13–6.
4. Meynardi F, Pasqualini ME, Rossi F, Dal Carlo L, Biancotti P, Carinci F. Correlation between dysfunctional occlusion and periodontal bacterial profile. J Biol Regul Homeost Agents. 2016;30: 115–21.
5. Avdeev OV, Drevnitska RO, Boykiv AB, Vydoinyk OY. Condition of fagocytosis of experimental animals with periodontitis due to modified reactivity. Wiad Lek. 2019;72:401–4.
6. Ebersole JL, Graves CL, Gonzalez OA, Dawson D III, Morford LA, Huja PE, et al. Aging, inflammation, immunity and periodontal disease. Periodontol 2000. 2016;72:54–75.
7. Boitsaniuk SI, Levkiv MO, Fedoniuk LY, Kuzniak NB, Bambuliak AV. Acute herpetic stomatitis: clinical manifestations, diagnostics and treatment strategies. Wiad Lek. 2022;75:318–23.

8. Rocca JP, Fornaini C, Wang Z, Tan L, Merigo E. Focal infection and periodontitis: a narrative report and new possible approaches. Int J Microbiol. 2020;2020:8875612.

9. Yussif NM, El-Mahdi FM, Wagih R. Hypothyrodism as a risk factor of periodontitis and its relation with vitamin D deficiency: mini-review of literature and a case report. Clin Cases Miner Bone Metab. 2017; 14:312–6.

10. Hasiuk N, Mazur I, Popovych I, Radchuk V. Clinical characteristics of diseases of the oral mucosa in patients who have undergone COVID-19 – What does a dentist need to know in a pandemic? Georgian Med News. 2021;10:93–9. Russian.

11. Zhang Y, Qiao D, Chen R, Zhu F, Gong J, Yan F. The association between periodontitis and inflammatory bowel disease: a systematic review and meta-analysis. Biomed Res Int. 2021;2021:6692420.

12. Maisonneuve P, Amar S, Lowenfels AB. Periodontal disease, edentulism, and pancreatic cancer: a meta-analysis. Ann Oncol. 2017;28:985–95.

13. Kothiwale S, Panjwani V. Impact of thyroid hormone dysfunction on periodontal disease. J Sci Soc. 2016;43:34–7.

14. Kochan O, Boitsaniuk S, Levkiv M, Przystupa K, Manashchuk N, Pohoretska K, et al. Emergence of nano-dentistry as a reality of contemporary dentistry. Appl Sci. 2022;12:2008.

15. Bergey DH. Bergey's manual of determinative bacteriology. Philadelphia (PA): Lippincott Williams & Wilkins; 1994.

16. Figdor D, Gulabivala K. Survival against the odds: microbiology of root canals associated with post-treatment disease. Endod Top. 2008;18:62–77.

17. Mendenhall WM, Sincich TL. Statistics for engineering and the sciences. 6th ed. Boca Raton (FL): Chapman & Hall; 2016.

18. Chen J, Su J, Kochan O, Levkiv M. Metrological software test for simulating the method of determining the thermocouple error in situ during operation. Meas Sci Rev. 2018;18:52–8.

19. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim). 2017;11:72–80.

20. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000. 2016;70:53–64.

21. Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. Periodontol 2000. 2016;70:65–79.

22. Sanara PP, Shereef M, Hegde S, Rajesh KS, Arun Kumar MS, Mohamed S. Comparative analysis of gingival crevicular fluid β-glucuronidase levels in health, chronic gingivitis and chronic periodontitis. J Pharm Bioallied Sci. 2015;7:S660–5.

23. Patini R, Staderini E, Lajolo C, Lopetuso L, Mohammed H, Rimondini L, et al. Relationship between oral microbiota and periodontal disease: a systematic review. Eur Rev Med Pharmacol Sci. 2018;22: 5775–88.

24. Monasterio G, Castillo F, Astorga J, Hoare A, Terraza-Aguirre C, Cafferata EA, et al. O-polysaccharide plays a major role on the virulence and immunostimulatory potential of *Aggregatibacter actinomycetemcomitans* during periodontal infection. Front Immunol. 2020;11:591240.

25. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30–44.

26. Chen C, Feng P, Slots J. Herpesvirus-bacteria synergistic interaction in periodontitis. Periodontol 2000. 2020;82:42–64.

27. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000. 2014;64:57–80.

28. Grover HS, Saini R, Bhardwaj P, Bhardwaj A. Immunity and periodontics: connecting the dots. Indian J Multidiscip Dent. 2015;5:62–7.

29. Feng Z, Weinberg A. Role of bacteria in health and disease of periodontal tissues. Periodontol 2000. 2006;40:50–76.

30. Aleksijević LH, Aleksijević M, Škrlec I, Šram M, Šram M, Talapko J. *Porphyromonas gingivalis* virulence factors and clinical significance in periodontal disease and coronary artery diseases. Pathogens. 2022; 11:1173.

31. Koliarakis I, Messaritakis I, Nikolouzakis TK, Hamilos G, Souglakos J, Tsiaoussis J. Oral bacteria and intestinal dysbiosis in colorectal cancer. Int J Mol Sci. 2019;20:4146.

32. Zaliznyak MS, Pohoretska HV, Levkiv MO. The dynamics of the periodontal pockets microflora of patients with osteoarthritis in the course of generalized periodontitis progression. Clin Dent. 2015;2: 38–42. Ukrainian.

33. Chornij N, Boitsaniuk S, Stechyshyn I, Pavliuk B, Manashchuk N, Chornii A, et al. Prevention and methods of correction of hyperesthesia of dental hard tissues of teeth. Pharmacologyonline. 2021;2: 1436–42.

34. Piasetska LV, Luchynskyi MA, Oshchypko RV, Rozhko VI, Luchynskyi VM. Evaluation of the treatment effectiveness of gingivitis by the oral dysbiosis index in patients with different reactions of psychophysiological maladaptation. Wiad Lek. 2020;73:2457–60.