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CLINICAL STUDY

The level of dysbiosis of the oral cavity depends on the type of dental prosthesis of the patient

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ABSTRACT

The work was aimed to determine the level of oral dysbiosis of patients depending on the type of prosthetics constructures.

MATERIALS AND METHODS: The study involved 48 patients with the presence in the oral cavity of fixed dentures from 4 to 6 units with a service life of no more than 3 years. The samples of plaque from the vestibular surface of dentures were collected to determine the microorganisms in the gingival plaque. Bacteriological research was carried out by the multiplex polymerase chain reaction in real time using the Phemoflor 8 reagent kit. The degree of dysbiosis of the oral cavity was determined according to the classification of V. Khazanova.

RESULTS: The results of the study of patients' samples did not reveal significant changes in the microbial landscape of the cervical areas. The total bacterial mass in the group of healthy individuals was lower than the total bacterial mass of patients in the investigated group significantly. IV degree of oral dysbiosis with a decrease in the number of lactobacilli, streptococci was characteristic of patients of denture wearers. The II degree of dysbiosis in patients with metal-ceramic structures was established. Patients who used solid cast and metal-plastic structures were diagnosed with II-III degree of dysbiosis of the oral cavity. The worst indicators were characteristic of prosthesis wearers of stamped-brazed structures.

CONCLUSIONS: Quantitative indicators of the composition of the microbiota of the cervical areas of denture wearers have significant differences and different levels of dysbiosis of the oral cavity, depending on the type of dentures in the patients' mouth *(Tab. 2, Fig. 1, Ref. 21).* Text in PDF *www.elis.sk*

Introduction

A meta-analysis of prospective and retrospective studies worldwide indicates the loss of at least one tooth per patient every 10 years, what means a loss of 0.1 teeth per person annually. Complete or partial tooth-loss has not only aesthetic consequences and represents psychological discomfort for the patient, but also contributes to the development of masticatory dysfunction due to anatomical changes in hard and soft tissues after tooth extraction. Therefore, such patients need complex rehabilitation with mandatory replacement of defects with prosthetics constructures. Reliable parameters for evaluating the effectiveness of prosthetics treatment are functionality, restoration of masticatory efficiency, and the absence of technical and biological complications. In turn, along with the improvement of the quality of life after the installation of prosthetics constructures, changes in the microbiota of the oral cavity are often observed in prosthesis wearers, which subsequently affects the health of the body as a whole. The dentures themselves are non-sterile and are placed on the border of the external environment - the human body, therefore they are in contact with both endogenous microorganisms of the oral cavity and exogenous microbiota actively. In addition to the fact that dental prostheses create additional places for the mechanical retention of microorganisms in the oral cavity, their construction materials are often an excellent substrate for the formation of bacterial biofilms. It has long been proven that the chemical composition of dentures affects the microbiocenosis and local immunity of the oral cavity significantly, what creates prerequisites for the deterioration of oral health. Therefore, the study of local and systemic changes in the body that occur after prosthetics with various dentures requires constant study and monitoring in order to improve the quality of prosthetics treatment of patients.

The study was aimed to determine the level of oral dysbiosis of patients depending on the type of prosthetics constructures.

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Materials and methods

Study population

The study involved 48 patients with an average age of 57 ± 6.0 years, who underwent prosthetics treatment for the replacement of tooth row defects at the Department of Prosthetics Dentistry and Implantology of the Poltava State Medical University (Ukraine) during 2021–2022. The inclusion criteria for patients were the presence in the oral cavity of fixed dentures from 4 to 6 units with a service life of no more than 3 years, subject to consent to participate in the study. The exclusion criteria were the absence of fixed prosthetics constructures in the oral cavity, the use of removable prostheses, the presence of fixed dentures of more than 6 units or with a service life of more than 3 years, pregnancy, diabetes, the presence of congenital or acquired immunodeficiencies, mental disorders, taking antibiotics the day before the collection of the material from the source of infection and refusal to participate in the study.

12 clinically healthy volunteers of the same age group (52 ± 4.5 years) with installed fixed dentures of 1–2 units or without prosthetics constructures in the oral cavity were included in the control group.

Written informed consent was obtained from each subject after a detailed explanation of the aims and protocol of the study, which was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans. The study was approved by the commission on biomedical ethics of Poltava State Medical University (minutes No 210 of November 23, 2022).

Determination of species and quantity composition of oral microbiota of patients

The samples of plaque from the cervical surface of the vestibular surface of dentures (crowns) in close proximity to the gingival margin (without touching or injuring it), on the upper and lower jaws were collected from the study participants to determine the microorganisms in the gingival plaque. A sample of dental plaque was collected using a sterile microbrush, which was placed in test tubes with a sterile saline solution and delivered within an hour to the laboratory of the Scientific Research Institute of Genetic and Immunological Basis of Development of Pathogenic Pharmacology (Poltava State Medical University, Poltava, Ukraine), where bacteriological research was performed. Bacteriological research was carried out by the multiplex polymerase chain reaction in real time (PCR-RT) using the Phemoflor 8 reagent kit (NPO DNA – Technology LLC, RU FSR 2009/04663).

Amplification results were recorded using a DT-322 detector amplifier (NPO "DNA Technology"), and the number of gene copies was calculated using the cycle indicator. Quantitative results were expressed in decimal logarithms.

The total bacterial mass and quantitative ratios of *Lactobacillus* spp., total *Enterobacteria* spp., *Streptococcaceae* spp., *Gardnerella* spp., *Prevotella* spp., *Porphyromonas* spp., *Eubacteria* spp., *Mycoplasma* (*hominis* + *genitalium*), and *Candida* spp. were determined.

The degree of dysbiosis of the oral cavity was determined according to the classification of V.V. Khazanova:

- dysbiosis shifts: the predominance of one type of opportunistic microorganism while preserving the normal composition of the oral microbiota;
- dysbiosis of the I–II degree: detection of two or three pathogenic agents against the background of a slight decrease in lactobacilli;
- dysbiosis of the III degree: a pathogenic monoculture is detected with a sharp decrease in the amount or in the complete absence of normal microbiota;
- IV degree dysbiosis: there are associations of pathogenic species of bacteria with yeast-like fungi.

Statistical analysis

Statistical analysis of the obtained results was carried out with the standard software packages "STATISTICA+" and "Microsoft Excel 2016". The presence of differences between the studied indicators was assessed by the Student's t-test. The results were considered reliable at values of $p < 0.05$.

Results

The results of the study of samples of patients with fixed prosthetics constructures using PCR-RF did not reveal significant changes in the microbial landscape of the cervical areas. Representatives of the *Enterobacteriaceae* genus and yeast-like fungi of the genus *Candida* were found in 100% of the examined individuals. In addition, lactobacilli, streptococci, eubacteria, gardnerella and prevotella were isolated from 96% of patients. It should be noted that *Mycoplasma* spp. were absent in the cervical microbiota of all patients participating in the study. That is, an increase in the frequency of isolation of anaerobic types of microorganisms was determined in patients who evolved into control and investigated groups, regardless of the presence of dentures in the oral cavity and their condition.

Despite the insignificant changes in the qualitative composition of the microbiota of the cervical areas of patients who use fixed dentures, we found significant changes in its quantitative

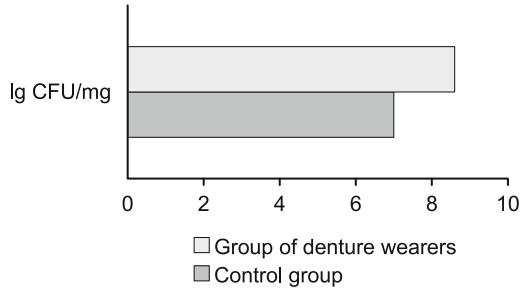

Fig. 1. Quantitative characteristics of the total microbial colonization of cervical areas, lg, CFU/mg (M ± m), (* probability of differences in indicators of the total bacterial mass with indicators of the control group, $p < 0.05$)

Tab. 1. Quantitative composition of the microbiota of cervical areas in examined patients, lg, CFU/mg (M ± m).

Microorganisms	Control group	Investigated group (denture wearers)
Lactobacillus spp.	4.5±0.88	1.72±0.6*
Enterobacteriaceae spp.	1.1±0.25	3.86±0.6*
Streptococcus spp.	6.8±0.74	5.64±0.34
Gardnerella. Prevotella	2.7±0.98	4.76±1.51*
Eubacterium spp.	2.5±1.1	5.02±0.67*
Candida spp.	1.2±0.44	4.03±0.52*

* reliability of the difference in values between indicator 1 and 2 of the examined groups, $p < 0.05$

composition compared to patients without prostheses. The total bacterial mass of cervical areas of patients of the control group was on average within 7.0 lg CFU/mg (Fig. 1). This indicator in the group of healthy individuals was significantly lower than the total bacterial mass of patients in the investigated group ($p < 0.05$). That is, the condition of prosthetics constructures and their availability influenced the indicators of the total bacterial mass in patients.

It is worth noting that the total bacterial mass of cervical areas of patients with more than 4 metal units exceeded the total bacterial mass of cervical areas of patients with 4 metal units. It means, the results of the study indicated an increase in the total bacterial mass of the cervical areas with an increase in the number of metal units in the patient's oral cavity.

Along with an increase in the total bacterial mass of cervical areas in patients with fixed dentures, our results indicated a violation of the microbiocenosis of these areas in all patients, regardless of the type of prosthetics constructure.

During the study, it was established that better indicators of the total microbial mass of stabilizing microorganisms of the oral cavity of patients without dentures or with 1–2 units of all-cast and metal-ceramic crowns (control group) were determined in comparison with the 2nd group of patients who participated in the study (Tab. 1).

The total microbial mass of *Streptococcus* spp. in patients of the group of denture wearers was lower, but it did not differ significantly from this indicator of patients without dental prostheses. In turn, the number of *Lactobacillus* spp. was significantly lower by 2.6 times compared to their number in patients of the control group ($p < 0.05$).

It is worth noting that the total microbial masses of representatives of the genera *Enterobacteriaceae*, *Eubacterium* and *Candida* were at a level that exceeded normal values. The number of enterobacteria in patients of the investigated group was 3.5 times higher than this indicator of the patients who made up the control group. The total microbial mass of representatives of the genus *Eubacteria* in the group of denture wearers was 5.02 ± 0.67 lg CFU/mg, what was twice as high as the indicators of patients without prothesis reliably ($p < 0.05$). The value of the number of yeast-like fungi *Candida* spp. in samples from the cervical areas of denture wearers averaged 4.03 ± 0.52 lg CFU/mg and exceeded the number of the specified microorganisms in patients of the control group 3.4 times and significantly ($p < 0.05$).

Taking into account the quantitative indicators of the microbiota of the cervical areas of patients of the control group, we established the II. degree of dysbiosis of the oral cavity according to the classification of V. Khazanova. At the same time, IV. degree of oral dysbiosis with a decrease in the number of lactobacilli, streptococci and the presence of opportunistic and pathogenic microorganisms in association with *Candida* spp. was characteristic of patients in the investigated group of denture wearers.

Characterizing the quantitative indicators of the composition of the cervical microbiota of dental wearers who had fixed prosthetics constructures of 4–6 units in the oral cavity, significant differences were found depending on the type of dentures of the patients (Tab. 2).

Thus, the smallest changes in the quantitative and qualitative composition of the microbiota of the cervical areas among patients with dentures were found in metal-ceramic prosthesis wearers. They were characterized by the preservation of stabilizing bacteria with the addition of a small number of pathogenic and conditionally pathogenic microorganisms. That is why, according to the classification of V. Khazanova we established the II degree of dysbiosis in patients with metal-ceramic structures.

We observed a decrease in *Lactobacillus* spp. in samples of individuals with solid cast as well as solid cast with plastic cladding constructions. However, the number of streptococci, which are part of the normobiota of the oral cavity, was within the normal range. Along with this, an increase in the number of *Enterobacteria*, *Prevotella* and *Candida* were found in the samples of these patients. Taking into account this fact, patients who used solid cast and metal-plastic structures were diagnosed with II–III degree of dysbiosis of the oral cavity.

The worst indicators were characteristic of prosthesis wearers of stamped-brazed structures. The IV. degree of oral cavity dysbiosis was revealed in the patients' data, which was characterized by a significant decrease in representatives of the genera *Lactobacillus* and *Streptococcus* against the background of an increase in the number of *Prevotella*, *Eubacteria*, and *Gardnerella* in association with yeast-like fungi.

Tab. 2. Quantitative composition of the microbiota of cervical areas in patients of dental wearers group with different prosthetics constructures. lg. CFU/mg (M ± m).

Microorganisms	Indicators of patients who used various dentures			
	Metal-ceramic	Solid cast with plastic cladding	Solid cast	Stamped-brazed structures
Lactobacillus spp.	4.1±0.86	1.64±0.57	3.45±0.82	3.17±0.74
Enterobacteriaceae spp.	2.4±0.41	4.7±0.63	3.72±0.25	5.4±0.58
Streptococcus spp.	6.6±0.23	6.6±0.32	5.8±0.33	4.43±2.73
Gardnerella. Prevotella	3.1±0.87	6.26±0.30	6.6±0.46	5.37±0.54
Eubacterium spp.	3.8±1.3	4.86±0.43	5.2±0.45	4.3±0.19
Candida spp.	1.8±0.88	3.28±0.19	3.52±0.07	3.7±0.12

Discussion

The oral cavity is a powerful site that is normally inhabited by more than 700 species of microorganisms, some of which cannot be cultivated (13). In healthy people with proper nutrition and sufficient oral hygiene, the microbiota coexists in symbiosis with the host, maintaining a balance that determines oral health as well as the body as a whole (14). Nevertheless, there are a number of factors which disturb the balance between bacteria and the host, what leads to a violation of the functions and health of the oral cavity – the so-called dysbiosis (14, 15). This contributes to a proportional decrease in the representatives of the stabilizing microbiota of the oral cavity, with subsequent colonization by pathogenic and opportunistic microorganisms, not characteristic of this site, and the formation of biofilms (14, 16, 17). It is worth noting that the formation of biofilms occurs both on the surface of hard and soft tissues of the oral cavity, and on artificial dental materials (composite fillings, veneers, removable and non-removable prosthetics structures, etc.). Moreover, the surface of prostheses or restorative materials itself, and sometimes the imperfection of the form, create additional retention points for microorganisms (18). Therefore, the results regarding increased microbial colonization of cervical areas in prosthesis wearers, compared to representatives of the control group, are natural.

In general, it is known that some microorganisms are able to form biofilms on the surfaces of prosthetic materials strongly. *Staphylococcus aureus*, *Candida albicans*, *Enterococcus* spp. and some species of *Stereptococcus* spp have the most significant potential in this regard (19). Undoubtedly, this leads to a shift in the quantitative and qualitative composition of the oral microbiota in denture wearers, characterized by a decrease in the total microbial portion of stabilizing microorganisms, such as lactobacilli and streptococci.

Along with this, literature data confirm the results obtained by us, that after the fixation of various fixed dentures, the gingival crevice is inhabited by various representatives of the oral microbiota (20). Thus, *C. albicans* is detected in significant numbers in the gingival crevices of patients with inflammation of periprosthetic tissues when using brazed structures (stainless steel) and cast prostheses (cobalt-chromium alloy), made according to the traditional techniques (21). In turn, the use of metal-ceramic structures demonstrates the best indicators of the qualitative and quantitative composition of the oral microbiota. According to the literature, remote studies of the microbiota of the gingival crevices of patients with metal-ceramic and metal-zirconium fixed prostheses 12 months after their fixation showed a decrease in the number of periodontogenic pathogens (20, 21). This, in fact, confirms the results of our research and explains the lowest degree of dysbiosis of the oral cavity precisely in patients whose adentia was replaced with metal-ceramic structures.

Conclusions

The total bacterial mass of cervical areas in patients with non-removable prosthetic structures from 4 to 6 units with a service life of no more than 3 years is significantly higher than the total bacterial mass of patients in the control group.

Better indicators of the total microbial mass of stabilizing microorganisms of the oral cavity in patients without dentures or with 1-2 units of all-cast and metal-ceramic crowns (control group), were determined in comparison with the studied group of patients.

Quantitative indicators of the composition of the microbiota of the cervical areas of denture wearers who had fixed prosthetics structures of 4–6 units in the oral cavity had significant differences and different levels of dysbiosis of the oral cavity, depending on the type of dentures in the patients' mouth.

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