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Evaluation of the State of Some Oral Obligate Anaerobic and Opportunistic Microflora by Periodontal Inflammatory Diseases

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Abstract –

Objective: The purpose of this research is to study the peculiarities of the state of oral microbiocenosis with the dominance of opportunistic microflora and to assess their prognostic significance as precursors for obligate anaerobes.

Methods and materials: The method of cultivation on immersion slides of Dentocult® LB (Finland) in the oral fluid has determined the presence of aerobic acidophilic bacteria (lactobacilli) and opportunistic microorganisms – yeast-like fungi of the genus Candida. Samples of dental plaque, oral fluid and periodontal pocket contents were examined using the polymerase chain reaction to determine the qualitative composition of periodontopathogens – Porphyromonas gingivalis and endodontalis, Tannerella forsythensis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum in young adults with clinically healthy periodontium, chronic catarrhal gingivitis and mild periodontitis.

Results: It has been established that the frequency of detection of obligate anaerobic flora – Porphyromonas gingivalis, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans – statistically significantly increased in the total microflora of the oral cavity tissues with the progressive development of chronic inflammation in the periodontal tissues against the background of the detection of their precursors – opportunistic lactobacilli and yeast-like fungi of the genus Candida.

Conclusion: The data obtained during this study have prognostic value not only for the early diagnosis of periodontal inflammatory diseases, but also for the development of effective therapeutic and prophylactic measures in young patients.

Keywords: periodontium, biotope, periodontopathogen, polymerase chain reaction.

INTRODUCTION

According to the World Health Organization (WHO), inflammatory periodontal diseases are among the most common diseases of the oral cavity, regardless of age, and are spread on average in 98% of population. One of the major etiological factors in the development of inflammatory diseases of periodontal tissue is considered to be the oral microflora promoting the development of inflammation in periodontal tissues, but specific types of microorganisms responsible for the occurrence of inflammation in periodontal tissues have not yet been identified [1-6]. Regardless of their degree of pathogenicity, interaction of various species of saprophytic and pathogenic microorganisms present in the oral cavity as a held biocenosis plays an important role in the mechanisms of development, maintenance and progression of pathological process in periodontal tissues. In this case, invasion of periodontopathogenic bacteria in the periodontal tissue is facilitated by primary adhesion to the surfaces of mucous membranes, and their fixation occurs due to the process of coaggregation. Precursors for coadhesion of periodontopathogenic Porphyromonas gingivalis and endodontalis, Tannerella forsythensis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum and Tannerella forsythia can be not only coccal flora consisting of facultative anaerobes and aerobes such as Streptococcus mitis, Streptococcus sangius, peptokokki, neisseria, Lactobacillus, but also opportunistic yeast-like fungi of the genus Candida [7-13]. Oral liquid promotes not only adhesion process, but also the process of elimination of microorganisms due to the buffer capacity of their environment and the content of glycoproteins and nonspecific protection factors – lysozyme, lactoferrin, sialoperoksidase and antimicrobial peptides. In inflammatory periodontal disease with severe gum bleeding, the microflora composition of the mouth is often dominated by microbial complexes consisting of periodontal pathogens having a great variety in the manifestation of not only their virulent properties, but also ability to colonize in the oral mucosa [14-16].

The characteristic clinical feature of chronic gingivitis and periodontitis with identifying representatives of obligate anaerobic and opportunistic microflora (periodontopathogenic, saprophytic Neisseria, yeast-like fungi of the genus Candida and lactobacilli) is that the originally observed asymptomatic clinical presentation with mild inflammation in periodontal tissues quickly evolves into the stage with marked clinical symptoms – the advent of increased bleeding, severe destruction, which results in resistance to treatment and requires the use of not only

modern highly effective diagnostic methods, but also complex treatment [2-3,7-8,17].

The purpose of the research is the assessment of certain types of obligate anaerobic and opportunistic microflora in the development of inflammatory periodontal diseases.

MATERIAL AND METHODS

Complex dental examination of 105 young patients aged 20 to 35 years without severe somatic pathology was carried out. The control group with clinically intact periodontium (CIP) consisted of 35 patients (15 men and 18 women) without complaints and visible pathological changes in the periodontal tissues, the 1st clinical group consisted of 35 patients with chronic gingivitis (15 men and 20 women), the 2nd clinical group included 35 patients with mild chronic periodontitis (MCP) (18 men and 15 women). All clinical groups were comparable by sex and age without significant somatic pathology.

The criteria for including patients in the clinical groups were the following: male and female people aged 20 to 35 years, indigenous residents of Ufa city, with the lack of quality therapeutic and prophylactic measures performed in the oral cavity for the last 6 months, complaints of bleeding when brushing teeth and eating hard food, who gave consent to participate in the study and agreed to its conditions. Clinical studies were conducted at the Department of Therapeutic Dentistry with the course of the Institute of Additional Professional Education of the Federal State Budget Educational Institution of Higher Education "Bashkir State Medical University" of the Ministry of Health of the Russian Federation. The survey was conducted using a technique that provided for an interview, obtaining the past medical history and disease history, an external examination and examination of the oral cavity. In the diagnosis of periodontal inflammatory diseases, evaluation of their severity and prevalence, the combined card recommended by WHO (1985) was used; the data of the interview and the examination, the results of additional research methods and the index assessment of the periodontal tissue condition – the papillary marginal alveolar index (PMA (1968), the simplified oral hygiene index OHI-S (Greene, Vermillion, 1969), the periodontal index PI (Russell, 1956), the sulcus bleeding index SBI (Muhlemann, Son, 1971) – were documented. The diagnosis was based on clinical results and X-ray (dental computed tomography).

To identify the presence of opportunistic microorganisms (lactobacilli and yeast-like fungi of the genus Candida) unstimulated oral fluid (saliva) for cultivation on Dentocult® LB submersible slides (Finland) was taken from all patients. Sowing of oral fluid was carried out on a special plate covered on both sides with a selective nutrient medium immersed in a container and incubated in a thermostat for 4 days at 37 °C. Subsequently, the immersion slide was compared with a sample and the amount of lactobacilli and yeast-like fungi of the genus Candida (in colony-forming units (CFU) in 1 ml of saliva) was determined.

To assess the status of obligate anaerobic flora a study of dental plaque, oral fluid and periodontal pocket contents using commercial DNA-express kits (LLC Research and Production Company (NPF) Litech, Russia) was conducted according to the instructions. Amplification of species-specific DNA fragments of microorganisms most often encountered in the periodontal inflammatory diseases - Porphyromonas gingivalis, P. endodontalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Tannerella forsythia - was carried out by polymerase chain reaction (PCR) using specific primers in a multichannel amplifier Tertzik MC-2 (NPF "DNA-Technology", Russia). The amplified DNA fragments were separated electrophoretically in a 2.0% horizontal agarose gel stained with ethidium bromide and visualized under ultraviolet illumination in the photodocumentation system.

Statistical processing of the data was carried out on a personal computer such as IBM PC/AT using the Statistica 7.0 application package and Excel 2007 spreadsheets. Based on the Student's t-test and the degree of freedom n, the probability distribution p was found from the distribution table t. Data for which the error probability (p) was less than 0.05 ($p < 0.05$) were considered reliable. For nonparametric data, the Biostat software package was used including the χ^2 criterion. The values ($p < 0.05$) were statistically significant.

RESULTS

As a result of a comprehensive dental examination, 66.7% of young people were diagnosed with severe clinical manifestations of periodontal inflammatory diseases. In 34.2%, of cases patients with healthy periodontics had latent inflammatory process. We have established that irrespective of the presence of the inflammatory process in the periodontal tissues, the level of oral hygiene in this category of patients was below the norm; therefore, the indices of the PMA, GI and SBI indexes ($p \leq 0.05$) significantly increased (Table 1).

Table 1: The most significant indicators of hygienic and periodontal indices of the control group and the group with chronic gingivitis (CG) and periodontitis (CP)

Indices indicators	Control group (CIP) (n = 35)	1st group CG (n = 35)	2nd group MCP (n = 35)
PMA index	10.7±0.03	29.8±0.03*	49.3±0.08**
OHI-S index	1.57±0.03	2.57±0.05*	2.92±0.01**
SBI (Müllmann)	0.45±0.05	1.48±0.05*	1.85±0.75**
Gingival index GI (Loe, Silness)	0.98±0.01	1.99±0.01*	2.6±0.01**
PI (Russell)	0.57±0.03	1.02±0.03*	1.52±0.05**

Note: * $p < 0.05$ – difference is statistically significant as compared to the control group
** $p < 0.05$ – difference is statistically significant by intergroup comparison

Table 2: The share of positive samples of detected opportunistic and obligate aerobic microorganisms in chronic gingivitis and periodontitis

Types of microorganisms	CIP (n = 35) plaque, oral fluid		Chronic gingivitis (n = 35) oral liquid		Chronic periodontitis (n = 35)contents of the periodontal pocket, oral fluid	
	abc	%	abc	%	abc	%
Lactobacilli	17	48.57	31	88.57*	23	65.71**
Candida spp. (C. albicans)	8	22.85	23	65.71*	22	62.86*
Treponema denticola	7	20	8	22.86	10	28.57*
Porphyromonas gingivalis	8	22.85	17	48.57*	18	51.42**
Porphyromonas endodontalis	4	11.43	8	22.86*	13	37.14**
Aggregatibacter actinomycetemcomitans	5	14.29	13	37.14*	16	45.71**
Fusobacterium nucleatum	12	34.28	17	48.57*	19	54.28**
Tannerella forsythia	4	11.43	6	17.14*	7	20*

Note: * p <0.05 – difference is statistically significant as compared to the control group
** p <0.05 – difference is statistically significant by subgroups comparison

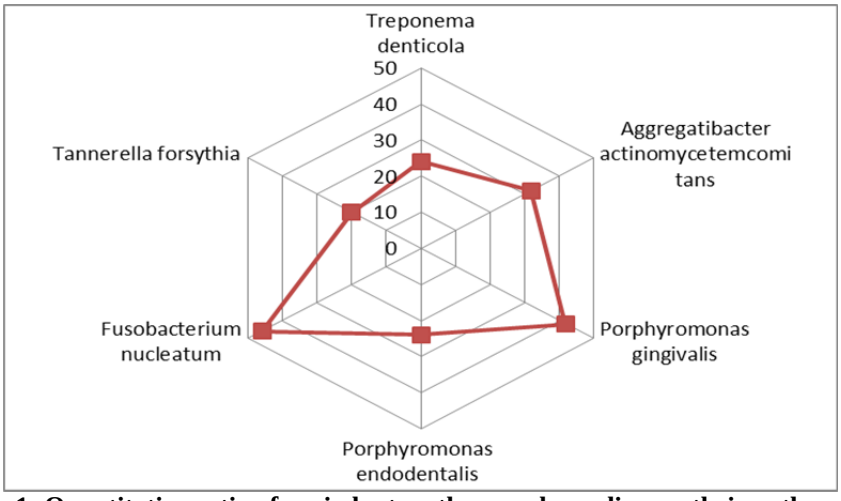

Figure 1: Quantitative ratio of periodontopathogens depending on their pathogenicity

In patients with CIP, chronic gingivitis and chronic periodontitis, associations of opportunistic microorganisms were most often identified. When analyzing associative relationships between lactobacilli and yeast-like fungi of the genus Candida, a significant excess of lactobacilli was obtained in oral fluid samples in chronic gingivitis than in the CIP ($\chi 2$ =12.99; p = 0.0003); in cases of chronic periodontitis and CIP there was no significant difference ($\chi 2$ = 2.10; p = 0.147); for opportunistic fungus of the genus Candida, a significant excess was found when comparing samples of oral fluid in case of chronic gingivitis and CIP ($\chi 2$ = 13.03; p = 0.0003) and chronic periodontitis and CIP ($\chi 2$ = 11.43; p = 0.001).

As a result of molecular genetic studies of plaque, oral fluid and the contents of the periodontal pocket by polymerase chain reaction, positive samples of specific DNA fragments of periodontopathogens were detected in most cases, both in CIP and with chronic gingivitis and periodontitis. In healthy periodontium Fusobacterium nucleatum were detected in 34.28% of cases, Porphyromonas gingivalis - in 22.85%, Aggregatibacter actinomycetemcomitans in - 14.29%. The occurrence of bacteria of these species is significantly higher in case of chronic gingivitis and periodontitis: Porphyromonas gingivalis were detected in 49% and 51.42% of cases, respectively; Fusobacterium nucleatum - in 48.57% and 54.28%; Aggregatibacter actinomycetemcomitans - in 37.14% and 45.71% (Table 2).

Regardless of the clinical state of periodontal tissues, all types of priority periodontopathogenic bacteria and opportunistic microflora were found in the biotopes under study.

Opportunistic microorganisms were frequently detected in the samples of oral fluid: lactobacilli and yeast-like fungi of the genus Candida were detected on average in 67.6% and 50.47% of cases, respectively. Their occurrence is significantly higher in cases of chronic gingivitis and periodontitis compared to the control group ($P \leq 0.05$).

In the studied biotopes, a combination of representatives of chromogenic complexes - such as Treponema denticola detected in 30.6% of cases, Porphyromonas gingivalis – in 23.8%, Tannerella forsythia – in 16.2% - on average in all clinical groups was noted in quantitative ratio which significantly correlates with the SBI and the PMA index, and indicates a risk of progression of inflammatory periodontal diseases ($p \leq 0.05$). Of the representatives of gram-negative bacteria, Fusobacterium nucleatum was also found on average in 45.7% of cases, Aggregatibacter actinomycetemcomitans – in 32.4% of cases, Porphyromonas endodontalis – in 23.8% of cases. The results are shown in Figure 1.

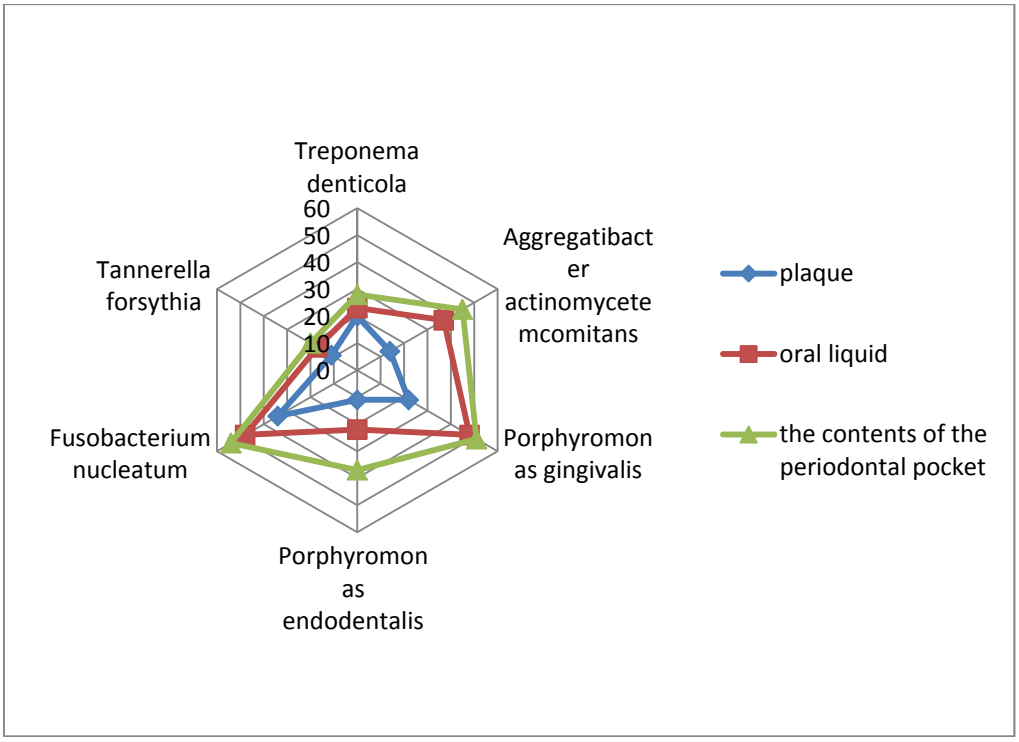

Figure 2: The frequency of occurrence of opportunistic and obligate-aerobic microorganisms in the test samples

When analyzing the results of dental plaque, oral fluid and the contents of the periodontal pocket obtained in the study depending on the clinical condition of the periodontal tissue, a correlation was established in the qualitative ratio of periodontopathogens ($P \leq 0.05$) (Figure 2).

In the molecular genetic study of biotope samples, periodontopathogenic bacterial species were detected in all patients ($p \leq 0.05$); their quantity was significantly variable – from 10^4 to 10^6 ml, for the representatives of opportunistic microflora on average from 10^3 to 10^6 CFUs/ml.

Discussion

The data of our studies showed that in young patients with CIP, chronic gingivitis and periodontitis of mild severity, a disturbance of the natural microbiocenosis of the oral cavity is observed with the identification of associations of opportunistic microflora – Lactobacilli and yeast-like fungi of the genus Candida - possessing a coadhesive ability for the development of obligate anaerobic microflora.

With the use of the PCR method, representatives of pigment-forming species of obligate anaerobic microflora prevail in the plaque samples – Fusobacterium nucleatum are found in 34.3% of cases, Porphyromonas gingivalis – in 23% of cases, Aggregatibacter actinomycetemcomitans – in 15%; in periodontal inflammatory diseases, their quantity increases not only in oral fluid samples, but also in the contents of the periodontal pocket: Porphyromonas gingivalis and Fusobacterium nucleatum are found in 50% of cases, Aggregatibacter actinomycetemcomitans and Porphyromonas endodontalis – in 40%.

Conclusion

1. Representatives of opportunistic microorganisms – lactobacilli and yeast-like fungi of the genus Candida - actively contributing to the process of coadhesion of periodontopathogenic microorganisms were identified in the examined oral fluid samples, irrespective of the clinical condition of the periodontium by culturing on the Dentocult® LB immersion slides (Finland).
2. Representatives of obligate-anaerobic microorganisms – Porphyromonas gingivalis, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans - which are markers of the development of the inflammatory process in the periodontal tissues, were most often detected by the PCR method in the biotopes under study.
3. The identification of the relationship between opportunistic and obligate-anaerobic microorganisms is of particular importance in the early diagnosis of inflammatory periodontal diseases and the planning of quality therapeutic and prophylactic measures in this category of patients.

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