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THE DEGREE OF INFLUENCE OF THE TYPES OF SELECTED MICROCULTURES FROM SUBGINGIVAL ZONE ON DIFERENT NOSOLOGICAL FORMS OF PERIODONTAL DISEASES IN YOUNG PEOPLE

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The development and course of periodontal disease in young people is characterized by sluggish clinical symptoms, and at the initial stage are asymptomatic, which greatly complicates its timely diagnosis. Therefore, it is especially important to find methods of early diagnosis and prediction of periodontal disease, which will allow for a rational comprehensive prevention. Among the causes of periodontal disease, the main role belongs to the microorganisms of dental plaque, which has important diagnostic value.

The aim of the study. *To investigate the composition of mixed microcultures of subgingival plaque bacteria in young people with different nosological forms of periodontal disease and to assess the degree of influence of microbial factors on the development of periodontal diseases.*

Materials and methods. *The state of microbiocenosis in the subgingival zone in 104 people with periodontal disease and in 94 people with intact periodontal tissues were studied. To assess the severity of the influence of the types of selected microcultures of the subgingival zone on the development and progression of periodontal disease in young people, the method of alternative sequential analysis of Wald and Bayes's formula was used.*

Results. *With the development of the inflammatory process in periodontal tissues in most cases revealed cultures characterized by the presence of polymorphic gram-negative bacteria that developed, fixed on the solid phase, and in the liquid phase contained coccal microflora (53.01±5.48 %). During the development of generalized periodontitis, an increasing number of microcultures with a predominance of gram-negative microflora with pronounced adhesive properties was detected, which led to the development of these microcultures on the solid phase (61,9±10,6 %). The calculation of prognostic coefficients showed that the presence of microcultures that developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci were highly important in terms of the development of periodontal disease (+7.46).*

Conclusions. *With the development of the pathological process in periodontal tissues, the state of the microflora changed in the direction of increasing gram-negative polymorphic microflora with pronounced adhesive properties that are capable of aggregation (up to 53.01 % in patients with catarrhal gingivitis, up to 90,48 % – with generalized periodontitis). The result of calculation of the severity of isolated types of microcultures from subgingival zone on the development and progression of periodontal disease in young people showed that the presence of microcultures that developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci were highly important in terms of the development of periodontal disease*

Keywords: *periodontal disease, young people, early diagnosis, subgingival microbiocenosis*

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1. Introduction

Periodontal diseases are one of the most common diseases of the oral cavity [1–3]. The development and course of periodontal disease in young people are characterized by sluggish clinical symptoms, and are asymptomatic in the initial stage, which greatly complicates its diagnostic and leads to late referral of patients to the dentist [1, 2, 4].

Periodontal diseases are considered as a pathological process caused by complex of bacterial agents, because of contravention of homeostasis between the subgingival microflora and the protective mechanisms of the body. Based on the research was formulated “The ecological hypothesis of dental plaque” as a basis of the etiopathogenesis of periodontal disease [5]. The etiology and pathogenesis of periodontal disease includes complicated relationship of etiological agents of dental plaque agents and different genetic and environmental risk factors, and their occurrence is often unpredictable [6]. The mechanism, which underlying in the basis of this dystrophic-inflammatory process includes both direct dam-

age of periodontal tissues by microorganisms of bacterial plaque and indirect damage by bacterial induction of the host, inflammatory and immune reactions [6].

Microorganisms of dental plaque, as a result of active secretion of various enzymes (hyaluronidase, chondroitinsulfatase, protease, catalase, etc.), have pronounced proteolytic activity. Bacterial proteolytic enzymes of dental plaque destroys the structural components of connective tissue, disrupt the microcirculation of periodontal tissues, cause a number of different inflammatory and immune reactions [6].

The number of authors notes the diagnostic value of microecological disorders of the oral cavity in predicting of the inflammatory gum diseases [7].

Approaches to the prevention and treatment of periodontal diseases should be based on local and general risk factors that play a leading role in their pathogenesis [8, 9]. Especially important is searching of methods for early diagnostic and prediction of periodontal diseases, which will allow rational comprehensive prevention.

The aim of the study. To investigate the composition of mixed microcultures of subgingival plaque bacteria in young people with different nosological forms of periodontal disease and to assess the degree of influence of microbial factors on the development of periodontal diseases.

2. Materials and methods

The study was conducted in the Department of Therapeutic Dentistry, Faculty of Postgraduate Education, of Danylo Halytsky Lviv National Medical University in 2016–2019.

The state of microbiocenosis in the subgingival zone was studied in 198 people 19–24 years old (104 people with periodontal diseases and 94 people with intact periodontal tissues). The gender ratio by sex in the studied was as follows: 77.27 % of men, 22.73 % – women.

The study was carried out in accordance with the Declaration of Helsinki, the Council of Europe Convention about human rights, biomedicine and relevant laws of Ukraine and approved by the Bioethics Commission of Danylo Halytsky Lviv National Medical University, the protocol No. 2 dated 16.11.2015. From all patients informed consent to participate in the study was received.

The material from the gingival groove or periodontal pocket was removed with sterile paper pin. The study was performed using standard polystyrene panels with 96 recesses. Subgingival plaque material was inoculated into incisions containing bacterial incubation environment and 10 mg of crushed tooth dentin. The incubation environment contained sterile filtrate of human saliva and liquid culture environments for different types of bacteria and microbial groups – Mueller-Hilton broth with glucose or the same broth with sheep blood. For isolation of *Aggregatibacter actinomycetemcomitans* was used environment with vancomycin, which inhibits the growth of coccal microflora. The plates were placed into desiccator with 5 % CO₂ and grow up to 5 days with daily growth control. The number of microbacteria that developed in the culture fluid (plankton phase) and in the solid phase, namely on the bottom, side surfaces and granules of crushed dentin was counted.

Based on the analysis of cultural and morphotinctorial properties, these cultures were divided into several types. Cultures of the first type grew mainly in the planktonic phase and contained coccal microflora with single gram-negative bacterias. The second type of cultures includes those that developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci. The third type of cultures was characterized by the presence of polymorphic gram-negative bacteria, which developed by fixation on the solid phase, and in the liquid phase contained coccal microflora. The fourth type of microcultures contained mainly a solid-phase form consisting of gram-negative bacteria that formed visible parietal groups *A. actinomycetemcomitans*, and also a small number of gram-positive cocci in the planktonic phase.

To assess the severity of selected microculture types of the subgingival zone on the development and progression of periodontal disease in young people, was used method alternative sequential analysis of Wald and calculation of the prognostic factor by mathematical processing of the obtained values by Bayes's formula.

Statistical processing of the study materials was performed on personal computer, using the statistical software package “Statistica 6.0” and “Microsoft Excel 2002”, by means of variation statistics methods. In the process of the results processing by the parametric method, were calculated the arithmetic mean of the variation series (M), the standard deviation (σ) and the mean error (m). Significance of difference of indicators was assessed using Student's t-test (t).

3. Results

It was established that the species composition of mixed bacteria microcultures of subgingival dental plaque depends on the nosological form of periodontal disease (Table 1).

During analyzing of the study results, it should be noted that in the selected microcultures, in young people without periodontal tissue lesions, were dominated cultures of the first type (93.62±2.52 %) – coccal flora with a small number of gram-negative bacteria in the planktonic phase. Only in singles cases were detected cultures of the second type (6.38±2.52 %), which were developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci. In contradistinction to the group of subjects with intact periodontal tissues, at presence of periodontal disease were dominated cultures of the third type (48.08±4.90 %), characterized by the presence of polymorphic gram-negative bacteria that were developed, fixed on the solid phase, and in the liquid phase contained coccal microflora and cultures of the second type (35.58±4.69 %). Also, were detected cultures of the fourth type contained mainly a solid-phase form consisting of gram-negative bacteria that formed visible parietal groups *A. actinomycetemcomitans*, and also a small number of gram-positive cocci in the planktonic phase (12.5±3.24 %), and in singles cases were detected cultures of the first type (3.85±1.89 %).

The difference between the indicators of the group of people with periodontal diseases compared to the group of people with intact periodontal tissues were statistically significant ($p < 0.001$).

In more than half of the examined people with catarrhal gingivitis (53.01±5.48 %) were found cultures of the third type, slightly less (42.17±5.42 %) cultures of the second type and very rarely (4.82±2.35 %) – of the first.

With the deepening of the pathological process in periodontal tissues and the development of generalized periodontitis from the subgingival area of the patients were isolated microcultures of the third (28.57±9.86 %) and fourth types (61.9±10.6 %) with a predominance of gram-negative microflora with pronounced adhesive properties, which led to the development of these microcultures on the solid phase (dentin granules, bottom and walls of the recesses of the plates for cultivation). Microcultures of dental plaque, which contained a solid-phase form, the basis of which were gram-negative bacterias, by morphological and cultural properties can be attributed to *A. actinomycetemcomitans*. Only in 2 patients (9,52±6,41 %) plaque bacterias developed in the incubation environment in the planktonic phase, but in addition to the coccal flora contained gram-negative rod-shaped bacteria and cocci (the second type of microcultures).

Table 1

The relationship between periodontal tissues condition and the types of isolated microcultures in examined patients (P±Sp)

Periodontal tissues condition			Types of isolated microcultures			
			1 type	2 type	3 type	4 type
Periodontal diseases	Totally n=104	abs.	4	37	50	13
		%	3.85±1.89***	35.58±4.69***	48.08±4.90***	12,5±3,24***
	Catarrhal gingivitis n=83	abs.	4	35	44	–
		%	4.82±2.35***	42.17±5.42***	53.01±5.48***	–
	Generalized peri- odontitis n=21	abs.	–	2	6	13
		%	–	9.52±6.41	28.57±9.86**	61,9±10,6***
Intact periodontal tissues n=94	abs.	88	6	–	–	
	%	93,62±2,52	6,38±2,52	–	–	

Note: significance of the difference between the indicators of groups of people with periodontal diseases and groups of people with intact periodontal tissues: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

The result of calculating of the severity of isolated types of microcultures from subgingival zone on the development and progression of periodontal diseases in young people showed that the prognosis of periodontal diseases, largely depends on the state of the microbiocenosis of subgingival plaque. The presence of microcultures of the second type (developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci) was of high importance in terms of the development of periodontal disease (+7.46). While the presence of microcultures of the first type (developed mainly in the planktonic phase and contained coccal microflora with single gram-negative bacteria) had a prognostic factor of -15.11, which indicated a low risk of disease.

4. Discussion

One of the main risk factors for periodontal diseases is the accumulation of pathogenic bacteria in dental plaque and homeostasis between the subgingival microflora and the body's defence mechanisms with the subsequent development of pathological processes in periodontal tissues [5–7]. In the isolated microcultures in individuals with intact periodontal tissues prevailed coccal flora with a small number of gram-negative bacterias in the planktonic phase (93.62±2.52 %) and in singles cases there were cultures that developed in the planktonic phase, but contained coccal microflora and gram-negative and cocci (6.38±2.52 %). With the development of the inflammatory process in periodontal tissues in most cases revealed cultures characterized by the presence of polymorphic gram-negative bacterias that developed, fixed on the solid phase, and in the liquid phase contained coccal microflora (53.01±5.48 %). During the development of generalized periodontitis, an increasing number of microcultures with a predominance of gram-negative microflora with pronounced adhesive properties was detected, which led to the development of these microcultures on the solid phase (61.9±10.6 %). According to the authors [10], the high frequency of isolated coccal microcultures with pronounced hemolytic properties indicated that they are the leading pathogenetic factors in the development of inflammatory periodontal diseases. The obtained results of the research confirm the data of the literature [7, 10] about the diagnostic value of micro-

ecological disorders in the oral cavity on periodontal diseases.

Existing studies proves the possibility of studying by mathematical methods of different relationships types between the representatives of the oral mycorbiocenosis in the dynamics of the intensity of the tooth decay process, which can have a prognostic effect on the conduct of hygienic and therapeutic procedures [11].

The calculation of prognostic coefficients showed that the presence of microcultures that developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci were highly important in terms of the development of periodontal disease (+7.46).

Study limitations. The study limitations should be considered the absence of the third and fourth type microcultures with a predominance of gram-negative microflora with pronounced adhesive properties, which led to the development of these microcultures on the solid phase in subjects with intact periodontium. The absence of such a restriction would allow to calculate the prognostic coefficients of influence of these types of microcultures on the development of and progression of periodontal diseases.

Prospects for further research. Further researches should be aimed at developing schemes for the prevention and treatment of periodontal diseases in the early stages, considering to the prognostic factors of their development.

5. Conclusions

1. With the development of the pathological process in periodontal tissues, the state of the microflora changed in the direction of increasing gram-negative polymorphic microflora with pronounced adhesive properties that are capable of aggregation (up to 53.01 % in patients with catarrhal gingivitis, up to 90,48 % – with generalized periodontitis).

2. The result of calculation of the severity of isolated types of microcultures from subgingival zone on the development and progression of periodontal disease in young people showed that the presence of microcultures that developed in the planktonic phase, but contained gram-negative rod-shaped bacteria and cocci were highly important in terms of the development of periodontal disease (+7.46). This indicated

that the prognosis of periodontal diseases, largely depends on the state of the microbiocenosis of subgingival plaque.

Conflict of interest

The authors declare that they have no conflict of interests.

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