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EVALUATION OF MARKERS OF PERIODONTAL DISEASE DEVELOPMENT ON BASED ON THE STUDY OF THE MICROBIAL COMPOSITION OF VARIOUS BIOTOPES OF THE ORAL CAVITY

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Annotation

Summary. The composition of supragingival and subgingival dental plaque, gingival and oral fluid, dentogingival or periodontal pocket, are a valuable diagnostic medium for non-invasive diagnosis of inflammatory periodontal diseases, since they contain a wide variety of microorganisms, many of which are markers.

Purpose — non-invasive assessment of markers of periodontal diseases based on the study of the microbial composition of various biotopes of the oral cavity by PCR.

Materials and methods. Non-invasive diagnosis risk factors, in particular, the presence of periodontal pathogenic microflora, provided for the study of various biotopes of the oral cavity — plaque, oral and gingival fluids, the composition of the dental pocket by applying a high-quality PCR diagnostic method using commercial DNA-express kits (LLC NPF «Litech», Russia).

Results. A comparative study of markers of the development of periodontal diseases using a qualitative version of the PCR test system in samples of subgingival plaque and dental pocket contents of individuals with initial stage I periodontitis showed 3.1 times greater prevalence of the representative of the yellow complex — Aggregatibacter actinomycetemcomitans, 1.4 times more often than the representative of the red complex P. gingivalis. In the studied material of supragingival plaque, gingival and oral fluid of individuals with gingivitis induced by dental biofilm, the representative of the yellow complex Aggregatibacter actinomycetemcomitans prevails 2.6 times more often than representatives of the red complex — P. gingivalis and Tannerella forsythia, 1.4 times the orange complex-Fusobacterium nucleatum. In samples of supragingival plaque in individuals with clinically healthy gums with loss of periodontal tissue, representatives of the microflora that form orange, red and yellow complexes most often predominate.

Conclusion. The study of supragingival and subgingival plaque, gingival and oral fluid, and the dentogingival pocket using a PCR test system allows timely detection of markers of the development of inflammatory diseases, regardless of clinical condition of periodontal disease.

Keywords: *biotopes, dental plaque, oral, gingival fluid, dentoalveolar pocket, markers, periodontal pathogens, polymerase chain reaction*

The authors declare no conflict of interest.

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ОЦЕНКА МАРКЕРОВ РАЗВИТИЯ ЗАБОЛЕВАНИЙ ПАРОДОНТА НА ОСНОВАНИИ ИЗУЧЕНИЯ МИКРОБНОГО СОСТАВА РАЗЛИЧНЫХ БИОТОПОВ ПОЛОСТИ РТА

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Аннотация

Актуальность темы исследования. Состав наддесневого и поддесневого зубного налета, десневой и ротовой жидкости, зубодесневого или пародонтального кармана является ценной диагностической средой при проведении неинвазивной диагностики воспалительных заболеваний пародонта, поскольку содержит большое разнообразие микроорганизмов, многие из которых являются маркерами.

Цель — неинвазивная оценка маркеров развития заболеваний пародонта на основании изучения микробного состава различных биотопов полости рта методом ПЦР.

Материал и методы. Неинвазивная диагностика факторов риска, в частности, наличия пародонтопатогенной микрофлоры предусматривала исследование различных биотопов полости рта: зубного налета, ротовой и десневой жидкости, состава зубодесневого кармана путем применения качественного метода ПЦР-диагностики с использованием коммерческих наборов ДНК-экспресс (ООО НПФ «Литех», Россия).

Результаты исследования. Сравнительное изучение маркеров развития заболеваний пародонта с использованием качественного варианта тест-системы ПЦР в пробах поддесневого зубного налета и содержимом зубодесневого кармана лиц с начальным пародонтитом стадии I показало в 3,1 раза большую распространенность представителя желтого комплекса Aggregatibacter actinomycetemcomitans, в 1,4 раза большую — представителя красного комплекса P. gingivalis. В исследуемом материале наддесневого зубного налета, десневой и ротовой жидкости лиц с гингивитом, индуцированным микробной биопленкой, в 2,6 раза чаще превалирует представитель желтого комплекса Aggregatibacter actinomycetemcomitans, соответственно в 2,1 и 1,4 раза чаще представители красного комплекса — P. gingivalis и Tannerella forsythia, в 1,4 раза оранжевый комплекс Fusobacterium nucleatum. В пробах наддесневого зубного налета, десневой жидкости у лиц с клинически здоровой десной при убыли тканей пародонта доминируют представители микрофлоры, формирующие оранжевый, красный и желтый комплексы.

Выводы. Исследование наддесневого и поддесневого зубного налета, десневой и ротовой жидкости, зубодесневого кармана с использованием тест-системы ПЦР позволяет своевременно выявить маркеры развития воспалительных заболеваний независимо от клинического состояния пародонта.

Ключевые слова: *биотопы, зубной налет, ротовая, десневая жидкость, зубодесневой карман, маркеры, пародонтопатогены, полимеразная, цепная реакция*

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The leading etiological factor of inflammatory periodontal diseases, according to domestic and foreign researchers, is the microflora of dental plaque, which later invades the gum tissue by overcoming the immunobiological barriers of the oral cavity. [1, 3, 12, 16, 19, 20].

Currently, when assessing the clinical condition of periodontal disease, practicing dentists most often use data from an objective examination, complaints, anamnesis, X-ray picture, which often does not give a complete picture of the etiology and pathogenesis of inflammatory diseases and does not allow you to accurately determine their course and prognosis. The requests of practicing dentists dictate the need to use reliable methods for detecting inflammatory periodontal diseases. Fast and objective diagnostics will help to significantly reduce the frequency of errors when making a diagnosis, will make it possible to monitor the dynamics of the effectiveness of treatment in real time. From the point of view of the etiology operiodontal diseases, the microbial composition of the supragingival dental plaque has a greater aggressiveness than the composition of subgingival plaque, not only due to the large variety of microorganisms, but mainly because of their properties-pathogenicity, virulence, adhesiveness and coadgesia. Microorganisms that form the composition of plaque are divided into two large groups: the first, cariesogenic, is formed from the following representatives — Streptococcus mutans, Lactobacillus acidophyllus, Str. Sobrinus. The second group is formed by periodontal pathogenic microorganisms — Streptococcus sanguis, Veionella parvulla, Streptococcus uberis, Porphyromonas gingivalis, Capnocytophaga ochracea, Rothia dentacariosa, Actinomyces viscosus, Phoprionibacterium aches, Praevotella intermedia, Fusobacteriae spirochetes, Veilonella alcoleusceus, Actinobacillus, etc. [1, 2, 5-10, 14, 17-19, 20].

In a healthy gingival furrow, the number of bacteria is insignificant, with a predominance of facultative gram-positive microorganisms in very small quantities, including periodontal pathogenic representatives — Porpyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitas, spirochetes. With the development of gingivitis in the supragingival plaque, there is a significant increase in the number of bacteria with a predominance of facultative gram-positive cocci, obligate gram-negative anaerobes, as well as the change of coccal flora by rod-shaped ones for example, in periodontitis, the number of facultative cocci, lactobacilli, obligate anaerobes, fusobacteria, protozoa and fungi of the genus Candida increases in the periodontal or periodontal pocket [1, 5, 13, 18, 20-25].

Dental plaque, oral and gingival fluids, and the composition of the periodontal or periodontal pocket are a unique biological environment for non-invasive diagnosis of periodontal diseases [3, 4, 8, 11, 15, 17]. In this regard, the task of timely detection of markers in the bacterial biocenosis of various species is extremely urgent. Biotopes of the oral cavity, which determined the relevance and purpose of our study.

Objective: to evaluate markers of periodontal disease development based on the study of the microbial composition of various oral cavity biotopes by PCR.

Material and methods of research

A comprehensive dental examination of 105 young people (from 20 to 35 years) using objective examination, analysis of complaints, assessment of hygienic and periodontal indices, X-ray examination allowed them to be divided into 3 clinical groups — with healthy periodontal tissues, gingivitis due to the influence of microbial biofilm, initial periodontitis stage I. Since in all clinical groups with clinicaly healthy gingiva with intact periodontium, gingivitis and periodontitis have revealed changes in hygiene and periodontal indices, we carried out non-invasive study of the microbiological status of the mouth that gave an opportunity to identify markers for the development and aggravation of diseases periodontal, and also to verify the clinical periodontal status, what consistent at the moment with the proposed criteria of the new International classification of periodontal diseases and the tissues around implants and the decisions of the world symposium of the American Academy of Periodontology (AAR) and the European Federation of Periodontologists (EPR) in 2017 [15].

The non-invasive study included the assessment and analysis of the microbiota state of the studied oral cavity biotopes-supragingival and subgingival plaque, gingival and oral fluid, as well as the contents of the dentogingival pocket using the method PCR. Study of the spectrum and number of periodontal pathogenic bacteria — Porphyromonas gingivalis, Pendodentalis, Treponema denticola, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Tannerella forsythia were performed using the method of PCR test system «Dentoscreen» (LLC of the Scientific Production Company (NPF) “Litech”, Russia) according to the instructions.

Statistical processing of the obtained data was carried out on a personal computer of the IBM PC/AT type using the Statistica 7.0 application software package and Excel 2007 spreadsheets based on the Student’s t-test value and degrees of freedom n, the probability of difference p was found from the distribution table t. Data for which the probability of error (p) was less than 0.05 were considered reliable ($p<0.05$). For nonparametric data, the Biostat software package was used, including the X2 criterion. $P<0.05$ was considered statistically significant.

The results of the study and their discussion

As a result of a comprehensive dental examination of individuals young age including analysis of the data of objective research, complaints, index assessment of periodontal tissues, radiographic parameters 82.87% cases diagnosed with marked clinical manifestations of chronic

inflammation, which helped to distinguish the group of individuals with a healthy periodontal status clinicaly gingival health jn a reduced periodontium, and the group with gingivitis- dental biofilm-induced, and with initial periodontitis stages I. In 17.13% of patients, during the examination, assessment of complaints, hygienic and periodontal indices, we diagnosed the state of clinical gingival heaith on intact periodontium (table 1).

When analyzing the data obtained by non-invasive study of the oral cavity biotopes-supragingival and subgingival plaque, gingival and oral fluid, dentogingival pocket, we found all types of priority periodontal pathogenic bacteria (table 2).

As a result of the analysis of samples of plaque, gingival and oral fluid content of the periodontal pocket using quality option test system PCR in most cases, the studies revealed a positive samples of specific fragments of DNA of periodontopathogenic microorganisms as in clinicaly healthy on a intact periodontium et all clinicaly healthy gingiva on a reduced periodontal, gingivitis, is caused by the influence of only the microbial biofilm and the initial periodontitis stage I.

By PCR in various oral cavity biotopes, regardless of the clinical low or critical titers of periodontal pathogenic microflora forming various complexes were found in periodontal tissues.

The most common microorganisms in parodontopathogenic the studied habitats, regardless of the diagnosed clinical condition periodontal were representatives of the red complex P. gingivalis from 22.85 percent to 71,42%, T. denticola from 20% to 28%, Tannerella forsythia from 11.43% to 20% of cases studies ($p < 0,05$).

When studying the prevalence of anaerobic representatives forming «red complex» obtained the following data. 3.1 P. gingivalis was frequently detected in subgingival dental plaque, periodontal pocket contents, oral fluid of persons with initial periodontitis of stage I compared with the studied biotopes of individuals with clinically healthy gums in intact periodontitis and individuals with gingivitis, dental biofilm-indused ($p < 0.05$). In a comparative study of samples of supragingival plaque, gingival and oral fluid, the titers of P. gingivalis is 2.1 times higher than the values of samples taken in individuals with clinically healthy on a redused periodontal ($p < 0.05$).

Comparative study galleries Tannerella forsythia showed that the contents of subgingival dental plaque and periodontal pocket, oral fluid of persons with early periodontitis stage I, respectively, 1.4 and 1.2 above

Table 1. Values of hygienic and periodontal indices in clinical groups of patients

Таблица 1. Значения гигиенических и пародонтальных индексов в клинических группах пациентов

Hygienic and periodontal indices	*Clinical gingival heaith on fn intact periodontium disease (n=27)*	*Clinical gingival heaith on a reduced periodontium (n=8)*	*Associated with dental biofilm alone (n =35)*	*Stage I: Intial Periodontitis (n = 35)*
Index — PMA	0	10,70 ± 0,03*	29,8 ± 0,03**	49,30 ± 0,08***
Index — OHI-S	0,7±0,01	1,57 ± 0,03*	2,57 ± 0,05**	2,92 ± 0,01***
SBI (Of Mulleman)	0	0,45 ± 0,05*	1,48 ± 0,05**	1,85 ± 0,75***
Gingival index GI (Loe, Silness)	0	0,98 ± 0,01*	1,99 ± 0,01**	2,60 ± 0,01***
Index Russell — PI	0	0,57 ± 0,03*	1,02 ± 0,03**	1,52 ± 0,05***

* *p <0.05 — the difference was significant with the difference was significant with the group with*
** *p <0.05*
*** *p <0.05– the intergroup difference is significant*

Table 2. The proportion of positive samples for detecting markers of periodontal disease development in the studied oral cavity biotopes

Таблица 2. Доля выявления положительных образцов маркеров развития заболеваний пародонта в исследуемых биотопах полости рта

Markers of the development of periodontal diseases (types of microorganisms)	*Clinical gingival heaith on a reduced periodontium (n=8) (supragingival dental plaque, gingival fluid)*		*Associated with dental biofilm alone (n=35) (supragingival plaque, gingival and oral fluid)*		*Stage I: Intial Periodontitis (n=35) (subgingival plaque, the contents of the periodontal pocket, oral liquid)*	
	абс.	*%*	*абс.*	*%*	*абс.*	*%*
Treponema denticola	7	20	8	22,86	10	28,57*
Porphyromonas gingivalis	8	22,85	17	48,57*	25	71,42**
Porphyromonas endodentalis	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**

* *p < 0.05 — the difference was significant with the difference was significant with the group with*
** *p < 0.05 — the intergroup difference is significant*

values compared to the studied biotopes of persons with clinically healthy gingiva with loss of periodontal tissues and those with gingivitis, caused by the influence of only a microbial biofilm (p<0.05).

In the contents of subgingival plaque and periodontal pocket samples oral fluid of persons with early periodontitis stage I periodontopathogen T. denticola were detected, respectively, 1.4 and 1.2 more than in the studied biotopes obtained from individuals with clinically healthy gingiva with intact periodontium, individuals with gingivitis, caused by the influence of only a microbial biofilm (p < 0.05).

Of the representatives forming the orange and green complexes most often in the studied habitats were found, Fusobacterium nucleatum, on average 34,28% to 54,28% of cases, Aggregatibacter actinomycetemcomitans from 14.29% to 45,71% of cases research.

Study of the titer frequency of microorganisms forming orange and green the complexes showed a prevalence of 3.2 and 1.2 times more frequently in the examined samples obtained from individuals with initial periodontitis stage I, 2.6 and 1.4 times higher values than in clinically healthy gingiva with loss of periodontal tissues, and 1.5 and 1.1 times more likely in biotopes obtained from individuals with gingivitis, associated with dtntal bofilm alone (p<0.05).

By PCR method depending on the revealed clinical condition of periodontal tissues in accordance with the proposed criteria of the new International Classification of periodontal diseases and tissues around implants and the decision of the World Symposium In 2017, the American Academy of Periodontology (AAR) and the European Federation of Periodontologists (EPR) revealed both low and critical titers of periodontopathogenic microflora in the studied oral cavity biotopes.

In 77.1 of cases of biotope studies by pcr we detected low titers periodontal pathogenic microflora in patients with clinically healthy gums with intact periodontitis, in 22.9% of cases of biotope studies in individuals with clinically healthy on a reduced periodontium tissue loss, critical titers of periodontal pathogenic microflora were detected.

In the studied biotopes of supragingival dental plaque, gingival fluid of persons with clinically healthy gingiva with intact periodontium 1.4 and 2.4 times more likely to determined low titers parodontopathogenic microflora compared to the studied habitats in individuals with gingivitis, caused by the influence of only a microbial biofilm and initial periodontitis stage I (p < 0.05).

In the group of individuals with gingivitis, caused by microflora microbial biofilm, have individuals with initial periodontitis stage I in the studied biotopes (oral fluid, subgingival plaque content of the periodontal pocket) by PCR revealed low titers of periodontal pathogenic microflora in 57.1 and 34.3% of cases, respectively, and critical titers in 42.9 and 65.7% of cases (p <0.05).

As can be seen from the data obtained by PCR in 65.7% of cases studies of individuals with the initial stage I periodontitis in the studied biotopes 2.9 times more often in composition subgingival plaque and the content of the periodontal pocket the high credits parodontopathogenic microflora, 1.5 times more frequently in the supragingival dental plaque, gingival and oral fluid of patients with gingivitis, due to the influence of only microbial biofilm (p <0.05), compared with the studied samples of supragingival plaque and clinical gingival healthy on a reduced periodontal.

Conclusions:

According to the conducted microbiological studies, low and critical titers of periodontal pathogenic microflora were detected in the studied oral cavity biotopes, regardless of their clinical condition, and their number was significantly variable, respectively, in the range of 104 up to 106 ml. From this it follows that these concentrations are sufficient both for the occurrence of inflammation, tissue loss in a clinically healthy gum, and for aggravation of inflammation in gingivitis and initial periodontitis.

In the comparative aspect of the study of markers of periodontal disease development using a qualitative version of the PCR test system in samples of subgingival plaque and the contents of the dental pocket in individuals with initial periodontitis of stage I, on average, 3.1 times more often prevail — Aggregatibacter actinomycetemcomitans, P. gingivalis, 1.7 times more common than Tannerella forsythia, 1.5 times more common Fusobacterium nucleatum and T. denticola compared to the studied biotopes of individuals compared to the studied biotopes of individuals with clinicaly gingival healthy reduced periodontal.

In the samples of subgingival plaque and the contents of the dental pocket of individuals with initial stage I periodontitis, P. gingivalis prevails 1.4 times and T. denticola, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, and Fusobacterium nucleatum are 1.2 times more common than in the studied biotopes of individuals with gingivitis dental biofilm industd.

The study material supragingival plaque, gingival and oral fluid of individuals with gingivitis, dental biofilm indused 2.6 showed the prevalence of Aggregatibacter actinomycetemcomitans, 2.1 times more likely P. gingivalis, and 1.4 times Tannerella forsythia and Fusobacterium nucleatum, 1.1 times more likely to T. denticola compared to the studied biotopes of persons with clinically gingiva healthy redusetd periodontal tissues.

In the biotope of supragingival plaque, gingival fluid of individuals with clinically healthy gums with periodontal tissue loss, representatives forming orange, red and yellow complexes prevail, the frequency of their occurrence is significantly lower than in gingivitis and initial periodontitis, however, the detected titers are sufficient for the development of inflammation and the process of bone destruction.

Thus, the study of biotopes of the oral cavity supragingival, subgingival plaque, gingival and oral fluid, the contents of the dentogingival pocket with using the pcr test system allows timely detection of markers of periodontal diseases.

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