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Frequency of Occurrence and Quantitative Content of Main Parodontopathogens at Periodontitis of Different Degree of Gravity

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Abstract

Aim of the study was analysis of occurrence of the main parodontopathogenic bacteria in the periodontal pockets with periodontitis of varying severity and depending on the tactics of patient management. It was found that in the case of chronic generalized periodontitis, the species of *Streptococcus mutans* were more frequently detected (by 40.6%, $p < 0.05$), *Streptococcus sobrinus* (by 39.1%, $p < 0.05$), *Streptococcus oralis* (31.4%, $p < 0.05$) and *Treponema denticola* (11.2%, $p < 0.05$) and associations *Treponema denticola-Porphyromonas gingivalis* (13.3%, $p < 0.05$). In severe cases we have obtained another results: *Porphyromonas gingivalis* ($p < 0.05$), *Streptococcus sobrinus* ($p < 0.05$), *Streptococcus salivarius* ($p < 0.05$), *Treponema denticola* ($p < 0.05$), *Streptococcus mutans* ($p < 0.05$), and *Streptococcus macacae* ($p < 0.05$). Uner the treatment by using Vector-therapy and antibacterial drugs, the detection rate of *Porphyromonas gingivalis* was significantly decreased by 18.6% ($p < 0.05$) and *Treponema denticola* by 15.1% ($p < 0.05$). The concentrations of *Porphyromonas gingivalis* (average concentration of 2.1E + 06 copies of DNA/ml) and *Treponema denticola* (average concentration of 1.2E + 06 copies of DNA/ml) were statistically significantly lower against the background of systemic antibiotic therapy under the using the pAL-TAStrSob16S calibrator designed by authors.

Keywords: *Periodontal Pocket; PCR; P. gingivalis; S. sobrinus; S. salivarius; T. denticola; S. mutans; S. macacae*

Introduction

Inflammatory pathology of periodontal disease takes second place in the world in terms of prevalence among dental diseases [1,2] and has no tendency to decrease [3]. In this case, microorganisms of the oral cavity have significant effect not only on the course of inflammatory process in periodontal tissues, but also for the course of somatic pathology [4-8].

In this regard, the characteristics of the microbiota periodontal waist pockets has great scientific and practical value, but remains a serious problem, due to on the one hand, physiological characteristics periodontopathogenic bacteria, and on the other - the absence standard reproducible teaching approaches for analysis of the contents of periodontal pockets. However, in recent years, thanks to more and more widespread use real-time polymerase chain reaction (PCR) time in dentistry, real the prospects for solving tasks [9] were methods for obtaining quantitative data are proposed about the microbiota of periodontal pockets [10]. This circumstance opens, in our opinion, fundamentally new the possibilities of ka for the diagnostic test of the etiological the importance of opportunistic periodontopathogens, so as to assess the effectiveness of the methods and/or conducted treatment.

Purpose of the Study

The purpose of the study is a comparative assessment of frequency occurrence and quantitative data on the content of major periodontopathogenic bacteria in the contents of the steam Dental pockets for periodontitis of varying degrees severity and depending on patient management.

Materials and Methods

The basis of the work is the results of a comprehensive survey 170 patients with periodontitis (main group), being on outpatient treatment at the State Healthcare Institution of the Republic of Belarus RB No. 2 (Ufa) in the period from 2012 to 2016. In 129 (75.9%) patients periodontitis of moderate severity was diagnosed, in 41 (24.1%) patients had severe periodontitis.

The control group was represented by 66 patients. (26 men and 40 women, average age 45.3 ± 7.62 years) without periodontal pathology after rehabilitation of the oral cavity. The diagnosis of the disease was established in accordance with criteria for the classification of periodontal diseases adopted at the meeting of the Presidium of the periodontology section Dental Association of Russia in 2001, based on International Classification of Diseases and Related Problems with health (ICD-10) [11].

All studies were ethical Helsinki standards and (2013) and Federal Law of the Russian Federation of November 21, 2011 № 323-FL "On the basics of protecting public health Russian Federation". Study Protocol Approved Ethics Committee of the Bashkir State Medical University. Each patient agreed to participate in the study and received detailed information about its results.

All patients of the main and control groups were performed molecular genetic research to for detecting periodontopathogenic species *Porphyromonas gingivalis, Treponema denticola, Streptococcus oralis, Streptococcus sanguis, Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus, Streptococcus macacae*, currently considered as the most informative "marker" periodontopathogens [6,12-14].

Material for molecular genetic research the contents of periodontal pockets of teeth.

The contents of the periodontal pocket were selected as follows way. Patients initially rinsed three times the oral cavity with physiological saline solution of chlorine chloride. Then, oral fluid was collected by spitting into a sterile tube of type Eppendorf (1.5 ml) and then introduced sterile paper endodontic forceps a pin (size No. 25) in the deepest sections of periodontal waist pockets for 10 s followed by placement into a sterile plastic tube like Eppendorf (1.5 ml), containing 1 ml of physiological saline. The fence was carried out in duplicate for each patient. Kept and transported the samples to the laboratory at + 4°C for 2 hours. Transportation of batches of samples to the laboratory. They were carried out in the temperature with a coolant genome. A molecular genetic study in patients was conducted twice - before and after 10 days of treatment as described scheme.

Bacterial DNA were isolated from 50 µl of clinical material (contents of periodontal pocket) using Chelex100 ion exchange resin.

For real-time PCR selected and tested species-specific pairs primers for studies with chambers DNA *Porphyromonas gingivalis, Treponema denticola, Streptococcus oralis, Streptococcus sanguis, Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus, Streptococcus macacae* and reaction. PCR Mix Mix SYBR Green I (SINTOL LLC). PCR was performed using a detecting amplification CFX96 Touch "REAL TIME" (Bio-Rad, USA). Profitability Analysis performed using software Bio-Rad CFX Manager. The instrument was calibrated with three dilutions calibration samples prepared by serial dilution of the plasmid pAL-TAStrSob16S known concentration.

Statistical processing of the results was performed using biometric analysis methods [15,16]. Descriptive statistics methods were estimation of arithmetic mean (M), average error average value (m) - for signs that have continuous distribution, as well as frequency of occurrence - for signs with a discrete value.

For the analysis of signs that obey the law of normal distribution, applied the method of detecting differences signs on average. Significance of Differences determined using t-student criterion. For analysis frequency distributions of signs that do not obey the law normal distribution, used criterion x2. For determine the relationship between the two features the correlation coefficient r was used, which was calculated Spearman nonparametric statistics method. The results were considered reliable at $p < 0.05$. Statistical processing of the obtained data was carried out using the software package Statistica 7.0, guided by allowance Trukhacheva N.V. according to statistical methods data processing in biology and medicine [17].

Results and Discussion

During the analysis of the frequency of occurrence of the main d about n that pa then gen about (*Porphyromonas gingivalis, Treponema denticola, Streptococcus oralis, Streptococcus sanguis, Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus, Streptococcus macacae*) [6] it was established that in patients with chronic generalized periodontitis in the contents of periodontal pockets were found all these types of bacteria. However, significantly more often, in compared with patients without periodontal disease in the group control, in patients with periodontitis outside of differentiation by severity, species *Streptococcus* were found mutans (by 40.6%, $p < 0.05$), *Streptococcus sobrinus* (by 39.1%, $p < 0.05$), *Streptococcus oralis* (by 31.4%, $p < 0.05$) and *Treponema denticola* (11.2%, $p < 0.05$) and among microbial associations in patients with periodontitis significantly increased co-occurrence species *Treponema denticola* and *Porphyromonas gingivalis* (by 13.3%, $p < 0.05$) when compared with the group comparisons.

In patients with severe disease in the contents periodontal pockets in descending order found significant increase, compared with that in healthy persons of representation *Porphyromonas gingivalis* ($p < 0.05$), *Streptococcus sobrinus* ($p < 0.05$), *Streptococcus salivarius* ($p < 0.05$), *Treponema denticola* ($p < 0.05$), *Streptococcus mutans* ($p < 0.05$) and *Streptococcus macacae* ($p < 0.05$) (Table 1).

Species	Before		After 14 days	
	Abs	%	Abs	%
Porphyromonas gingivalis	21	24.4	5 [a]	5.8
	p = 0.001			
Treponema denticola	18	20.9	5 [a]	5.8
	p = 0.005			
Streptococcus mutans	58	67.4	41 [a]	47.7
	p = 0.017			
Streptococcus salivarius	9	10.5	5	5.8
	p = 0.373			
Streptococcus sanguis	53	61.6	35 [a]	40.7
	. p = 0.011			
Streptococcus oralis	48	55.8	28 [a]	32.6
	. p = 0.002			
Streptococcus macacae	10	11.6	6	7.0
	. p = 0.436			
Streptococcus sobrinus	47	54.7	24 [a]	27.9
	$\chi^2 = 12,68$, p = 0,001			

Table 1: *The frequency of allocation of opportunistic bacteria in the contents of the periodontal pocket in patients with periodontitis of the main group (n = 86).*

Note: [a]: The difference with the value before treatment is significantly ($p < 0.05$).

According to research, in patients with periodontitis moderate severity statistically significant differences in the frequency of bacteria compared to the control group revealed only for *Streptococcus mutans* ($p < 0.05$), *Streptococcus sobrinus* ($p < 0.05$) and *Streptococcus oralis* ($p < 0.05$). Comparative analysis of molecular genetic data studies of the contents of periodontal pockets of patients with varying degrees of severity of periodontitis allowed to reveal that differences are characteristic for the same x patients as a part of m and microbiocenosis of periodontal pockets. It was found that in patients with severe periodontitis in comparison with a group of patients with an average severity significantly more common species *Streptococcus macacae* ($p < 0.05$), *Streptococcus salivarius* ($p < 0.05$), *Streptococcus sanguis* ($p < 0.05$), *Streptococcus oralis* ($p < 0.05$) and *Porphyromonas gingivalis* ($p < 0.05$).

Analysis of social and social networks periodontopathogenic and about periodontal disease of different severity.

According to the literature [3,18,19], it is known that bacteria subbiotopes of the oral cavity, in particular periodontal pockets most commonly represented in microbial associations, formed by obligate and facultative anaerobic critical microorganisms in the aggravation of the inflammatory process in periodontal tissues. Accordingly, the analysis of the presence of associative relations between periodontopathogenic microorganisms in patients periodontitis is an important diagnostic tool when monitoring and predicting the course diseases, in determining tactics for further management the patient. That is why we conducted an analysis on subject of the presence of associative relationships between periodontopathogenic species in patients with moderate to severe

severity of periodontitis.

In patients with moderate periodontitis in comparison with a group of patients with intact periodontal contents periodontal pockets significantly more often than others associations of *Streptococcus mutans, Streptococcus sanguis, Streptococcus oralis* ($p < 0.05$), *Porphyromonas gingivalis, Treponema denticola* ($p < 0.05$), *Streptococcus mutans, Streptococcus sanguis, Streptococcus oralis, Streptococcus sobrinus* ($p < 0.05$). In patients with severe periodontitis was observed significantly higher than in patients with periodontitis moderate and in healthy individuals, the frequency of occurrence associations of microorganisms *Porphyromonas gingivalis, Treponema denticola* ($p < 0.05$), *Streptococcus mutans, Streptococcus sanguis, Streptococcus oralis* ($p < 0.05$).

Qualitative assessment of periodontopathogenic m and to r about r g of n and zm about in k l and n and with to x x about b rats and x. Against the background of the treatment in patients, ka to the main groups, such as comparison groups, a certain decrease in the representation of all studied periodontal pathogens. In the main group of patients during complex therapy, including Vector-therapy and antibacterial drugs, attention is drawn to significant decrease in detection frequency in content periodontal pockets *Porphyromonas gingivalis* by 18.6% ($p < 0.05$) and *Treponema denticola* by 15.1% ($p < 0.05$) (See table 1).

In patients of the comparison group for which Vector therapy is not appointed, there was a significant decrease in frequency discharge in the contents of the periodontal pocket *Streptococcus mutans*- by 16.6% ($p < 0.05$), *Streptococcus sanguis* - by 16.6% ($p < 0.05$), *Porphyromonas gingivalis* - by 13.1% ($p < 0.05$) and *Treponema denticola* - by 11.9% ($p < 0.05$) (Table 2). Changes in the content of other species were not significant. So, if in the main group the frequency of representation *Streptococcus oralis* and *Streptococcus sobrinus* reliably decreased compared to levels preceding treatment, then in the comparison group changes in the frequency of detection the bacteria were inconsequential. At this inclusion in the treatment of periodontitis complex treatment apparatus Vector contributed to a more significant decrease in prevalence of *Streptococcus oralis* and *Streptococcus sobrinus* in the contents of the periodontal pocket respectively 23.2% ($p < 0.05$) and 26.8% ($p < 0.05$).

In studying the dynamics of changes in microbiota in the contents periodontal pocket depending on the degree severity of periodontitis in both groups of patients established significant decrease in the representation of most microorganisms (Table 3). So, in patients of the main group with periodontitis of moderate severity *Porphyromonas gingivalis* was lower than before treatment start at 15.6% ($p < 0.05$) and *Treponema denticola* - by 12.5% ($p < 0.05$). Among the representatives genus *Streptococcus spp*. most pronounced changes were found for species *Streptococcus sobrinus* - decrease by 31.3% ($p < 0.05$), *Streptococcus oralis* - by 28.1% ($p < 0.05$), *Streptococcus sanguis* - 23.5% ($p < 0.05$) and *Streptococcus mutans* - by 20.3% ($p < 0.05$). Periodontopathogen release rate *Streptococcus salivarius* and *Streptococcus macacae* remained virtually unchanged, however, it should be noted that their occurrence before treatment among this contingent patients was low and did not exceed 6.3%.

Species	Before		After 14 days	
	Abs	%	Abs	%
Porphyromonas gingivalis	20	23.8	9 [a]	10.7
	p = 0.040			
Treponema denticola	14	16.7	4 [a]	4.8
	p = 0.023			
Streptococcus mutans	60	71.4	46 [a]	54.8
	.p = 0.033			
Streptococcus salivarius	10	11.9	5	6.0
	.p = 0.277			
Streptococcus sanguis	45	53.6	31 [a]	36.9
	. p = 0.036			
Streptococcus oralis	44	52.4	36 [a]	42.9
	. p = 0.279			
Streptococcus macacae	9	10.7	6	7.1
	. p = 0.626			
Streptococcus sobrinus	40	47.6	31	36.9
	. p = 0.241			

Table 2: *The frequency of allocation of opportunistic bacteria in the contents of the periodontal pocket in patients with periodontitis comparison group (n = 84)*

Note: [a]: The difference with the value before treatment is significantly (p < 0.05).

Parameters	Main group		Comparison group	
	Before	After 14 days	Before	After 14 days
Periodontitis of moderate severity				
Number of patients	64		65	
Porphyromonas gingivalis	14 (21.9%)	4(6.3%) [a]	12 (18.5%)	6 (9.2%)
Treponema denticola	10 (15.6%)	2 (3.1%) [a]	9 (13.8%)	2 (3.1%)[a]
Streptococcus mutans	47 (73.4%)	34 (53.1%)[a]	49 (75.4%)	37 (56.9%)[a]
Streptococcus salivarius	4 (6.3%)	3 (4.7%)	4 (6.2%)	1 (1.5%)
Streptococcus sanguis	44 (68.8%)	29 (45.3%)[a]	38 (58.5%)	25 (38.5%)[a]
Streptococcus oralis	39 (60.9%)	21 (32,8%)[a]	38 (58,5%)	31 (47.7%)
Streptococcus macacae	3 (4.7%)	2 (3.1%)	5 (7.7%)	3 (4.6%)
Streptococcus sobrinus	38 (59.4%)	18 (28.1%)[a]	33 (50.8%)	26 (40.0%)
Severe periodontitis				
Number of patients	22		19	
Porphyromonas gingivalis	7 (31.8%)	1 (4.5%) [a]	8 (42.1%)	3 (15.8%)[a]
Treponema denticola	8 (36.4%)	3 (13.6%)[a]	5 (26.3%)	2 (10.5%)
Streptococcus mutans	11 (50.0%)	7 (31.8%)	11 (57.9%)	9 (47.4%)
Streptococcus salivarius	5 (22.7%)	2 (9.1%)	6 (31.6%)	4 (21.1%)
Streptococcus sanguis	9 (40.9%)	6 (27.3%)	7 (36.8%)	6 (31.6%)
Streptococcus oralis	9 (40.9%)	7 (32.8%)	6 (31.6%)	5 (26.3%)
Streptococcus macacae	7 (31.8%)	4 (18.2%)	4 (21.1%)	3 (15.8%)
Streptococcus sobrinus	9 (40.9%)	6 (27.3%)	7 (36.8%)	5 (26.3%)

Table 3: *The frequency of bacteria in the contents of the periodontal pocket in patients with moderate to severe periodontitis, depending on the treatment tactics.*

Note: [a]: The difference with the value before treatment is significantly (p < 0.05).

In patients of the main group with a severe form of the disease in the contents of periodontal pockets authenticity different differences with indicators before treatment were identified for *Porphyromonas gingivalis* and *Treponema denticola* - below respectively by 27.3% ($p < 0.05$) and 22.8% ($p < 0.05$). It should be noted a significant decrease in representation in the contents of the periodontal pocket *Streptococcus mutans* (by 13.6%, $p < 0.05$), however these differences were not statistically significant and wore trends. For patients of the comparison group with an average degree periodontitis in the contents of the periodontal pocket significant decrease in bacteria *Streptococcus sanguis* - by 20.0% ($p < 0.05$), *Streptococcus mutans* - by 18.5% ($p < 0.05$) and *Treponema denticola* - by 10.7% ($p < 0.05$).

In patients of this group with a severe form of the disease periodontitis noteworthy decrease the occurrence of *Porphyromonas gingivalis* (26.3%, $p < 0.05$) and *Treponema denticola* (21.1%, $p < 0.05$), however, these differences with the initial indicators turned out to be unreliable, which is probably due to a small sample size (19 people). In the same time total decrease in representation of the indicated microorganisms was statistically significant - by 47.4% ($p < 0.05$). Thus, conducting molecular genetic studies in patients with periodontitis allowed to evaluate bacteriostatic effect of the treatment and to prove the feasibility of inclusion in the composition of the basic antibacterial ultrasound treatment of the disease gingival pockets and root surfaces using apparatus Vector.

The quantitative assessment of the periodontopathogenesis. Quantification of conditionally pathogenic content microorganisms is one of the most widely used ways to assess their etiological significance, mandatory for the diagnosis of infectious and inflammatory processes, including in periodontal tissues. However, for obtaining the indicated quantitative data by PCR in real time with periodontitis to the present certain technical difficulties existed standardization studies due to the difficulty of extracting and measuring the amount of content periodontal pockets. In this regard, we have developed a method for obtaining clinical samples of known volume [10], as well as with designed pAL-TAStrSob16S calibration sample with known concentration (copies of DNA/ml) to obtain reliable results in case of periodontitis. The quantitative content of bacteria in equalized the volume of clinical samples was determined by PCR in real-time instrument calibrated three dilutions of the recombinant plasmid pALTAStrSob16S, which allowed to determine the absolute amount copies DNA of the pathogen in the clinical sample (copies DNA/ml).

In the group of patients who underwent a course of systemic antibiotic tick therapy, a statistically significant decrease was observed concentrations of periodontopathogens *Porphyromonas gingivalis* (average concentration - 2.1E + 06 copies of DNA/ml) and *Treponema denticola* (average concentration - 1.2E + 06 copies DNA/ml) (Table 4).

Species	Main group		Comparison group	
	Before treatment	After 10 days	Before treatment	After 10 days
Porphyromonas gingivalis	1.1E+07	5.7E+06[a]	7.2E+07	2,1E+06[a]
Treponema denticola	2.5E+07	4.8E+06[a]	6.3E+07	1,2E+06[a]
Streptococcus oralis	2.4E+08	9.7E+07[a]	3.8E+08	4,7E+07
Streptococcus sanguis	4.3E+08	1.7E+06[a]	4.7E+08	4,7E+06
Streptococcus sobrinus	4.4E+08	8..8E+06[a]	5.4E+08	2.1E+06
Streptococcus mutans	1.6E+09	7.7E+07[a]	2.7E+09	3.9E+07
Streptococcus salivarius	4.1E+08	1.3E+06[a]	1.2E+09	8.7E+06
Streptococcus macacae	3.5E+09	7.9E+07[a]	7.3E+07	3.5E+06

Table 4: *The absolute number of pathogenic and conditionally pathogenic bacteria in the contents of periodontal pockets in patients with chronic generalized periodontitis (DNA copies/ml). Note: [a]: significance of differences in treatment ($p < 0.05$).*

Conclusion

The concentrations of *Porphyromonas gingivalis* (average concentration of 2.1E + 06 copies of DNA/ml) and *Treponema denticola* (average concentration of 1.2E + 06 copies of DNA/ml) were statistically significantly lower against the background of systemic antibiotic therapy under the using the pAL-TAStrSob16S calibrator designed by authors. Thus, obtained by using molecular genetic methods quantitative data about the species composition of periodontopathogenic bacteria allow to some extent objectively evaluate the effectiveness treatment (in our case, ultrasound) and microbiological features of chronic generalized periodontitis of various degrees of severity, increase the validity of antibiotic therapy and, accordingly, reduce risks of selection of antibiotic-resistant options. Continued research in the designated area will contribute to the accumulation of data to obtain statistically significant values, necessary for the development of reference intervals and their inclusion in relevant protocols and / or standards providing medical care to patients with this pathology.

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