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Monitoring of the microbial community of periodontal pockets in patients with chronic generalized and aggressive periodontitis

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Abstract: The fundamental problem of general medicine and particularly of periodontology, remains the accurate and early diagnostics, which allows to start the casual treatment as soon as possible. The panel test-system, developed earlier, is based on the real-time PCR system for the qualitative and quantitative analysis of the oral microbial communities considering the microorganisms associated with periodontal diseases. The panel allows the simultaneous quantitative determination of these periodontal pathogens with the diagnostic accuracy of not less than 97 %. The study included 78 patients with clinically and radiographically confirmed diagnosis of chronic generalized periodontitis of moderate and severe degrees and patients with aggressive periodontitis.

Keywords: Microbiocenosis, Chronic Generalized Periodontitis, Antibioticotherapy, Real-Time PCR, QPCR

1. Introduction

Human microbiome investigation is one of the fast developing fields of systemic biomedicine. Residential microbial population is evolutionary elaborated and physiologically essential component, which fulfils some basic metabolic functions and protects from microbial invasion. Taxonomic composition of the human microbiota depends on multiple factors, including ethnic, physiological, genetic, sociocultural, behavioural, dietary regime. Microbiotic changes indicate both the health status and several illnesses and pathologies.

Bacteria composing the normal microbial community could be divided into 3 groups: 1) residential bacteria, 2) opportunistic pathogenic bacteria, 3) pathogenic bacteria [1]. Stable normal microbial community expels the various pathogens form the microbiocenosis and reduces the chances for infection when pathogen reaches the human body [2, 3, 4].

Much attention is given to the oral microbial community analysis lately. The mucosa plays a unique role in the human-environment interaction. The oral cavity is a complex ecological system, which consists of viruses, bacteria, fungi and protozoa [2, 3, 4]. More than 700 species are to be found in such biotopes as saliva, gingival fluids, periodontal pocket and biofilm [5,6].

Quantity and species composition of oral microflora in each healthy individuum is considerably stable due to several factors assuring it. One of the main roles plays the antagonism of residential microorganism towards the opportunistic flora [7, 8].

Disbalance in residential and opportunistic flora leads to the disbiotic disturbances and is characterised by decrease in relative amount of lacto- and bifidobacteria. One of the clinical implications of this disbalance could be such a highly widespread disease as periodontitis.

Periodontal diseases are thought to have a polybacterial nature, with each of them having its own periodontal pathogenic profile [8]. Despite of a wide microorganism pallet found in the periodontal pockets, only a few of them are considered to be periodontal pathogens: *Actinobacillusactinomycetemcomitans, Porphyromonasgingivalis, Bacteroidesforythus, Prevotellaintermedia, Eicenellacorrodens, Fusobacteriumnucleatum*и*Treponemadenticola* [7]. These

pathogens are subdivided into invariable bacterial complexes specific for clinical conditions of the oral cavity [8].

The panel of real-time PCR test systems, designed earlier for quantitative and qualitative analysis of microbial communities of oral biocenosis considering the microorganisms associated with periodontal diseases, allows to fulfill the simultaneous quantitative specification of the periodontal pathogens with the diagnostic accuracy not less than 97% [Zorina O.A. 2011].

2. Main Body

2.1. The Aim

The aim of this study was to increase the diagnostic efficacy for chronic generalized and aggressive periodontitis by means of quantitative evaluation of the most important periodontal pathogen species using real-time PCR.

2.2. Materials and Methods

78 patients (37 male and 41 female) aged between 18 and 60 years having chronic generalised periodontitis (CGP) of moderate and severe levels or having aggressive periodontitis. Patients were divided into 3 groups: 1. Moderate CGP (35 patients), 2.Severe CGP (22 patients), 3.Aggressive periodontitis (22 patients). The control group included 20 almost healthy patients with intact periodontium aged between 18 and 35 yrs.

Patients underwent the standard dental observation, medical chart was filled in, form and level of the disease was defined according the inflammation signs, destruction of the periodontal tissues, exudation from periodontal pockets, gum bleeding during the tooth brushing.

Periodontal depth was measured around each tooth on four sides: medial, distal, oral and vestibule with the periodontal probe Goldman-Fox (Hu-Friedy Mfg. Co. Inc., USA).

Sterile paper points ISO 25 were used for sampling. We have used previously designed test-system which consists of specific primers and fluorescently labelled probes specific for six periodontal pathogens (*Aggregatibacteractinomycetemcomitans*, *Porhpyromonasgingivalis*, *Prevotellaintermedia*, *Tanerellaforsythensis*(*Bacteroidesforsythus*), *Treponemadenticola*, *Candida albicans*). The real-time PCR was performed using detection thermal cycler "DT-96" (DNA-Technology LLC, Russia). Amplification results were analyzed using the software provided by the thermal cycler manufacturer.

2.3. Results and Discussion

According the achieved data the total subgingival bacterial mass in patients with intact periodontium was 10^6 genome-equivalents per reaction test tube, whereas in patients with CGP this index was 2-3 periods higher and totalled 10^7 genome equivalents per reaction test tube in the first and 10^8 genome equivalents per reaction test tube in the second and third group.

As a result of PCR analysis we have received the data on the incidence (in %) of the main periodontal patogenes in subgingival fluid sampled from patients with CGP, aggressive periodontitis and the control group. (Tab. 1)

As seen in the Table 1 the percentage of positive samples increased with the progress of disease. Among the periodontal pathogens the most often ones were such DNA markers as*P.intermedia*, *T.forsythensis*, *T.denticola.* In severe CGP the incidence of *P.gingivalis* and*C.albicans*was increased. In comparison with control group the patients with CGP showed less unique DNA markers: in moderate CGP more often were the groups of 2-4 markers, in severe one – 3-5. This indicates the possible synergic collaboration of different periodontal pathogens.

A.actinomycetemcomitans is the most common in the periodontal pockets in patients with aggressive periodontitis and could be found in 70,0% cases. This value was 1,5 fold more, than in patients with CGP and 7 fold more than in intact periodontium cases.

Table 1. *Positive samples rate in groups (%)*

Microorganism	Control Group (n=100)	Moderate CGP (n=100)	Severe CGP (n=100)	Aggressive Perioodntitis
A.actinomycetemcomitans	10,0	48,0	43,0	70,0
P.gingivalis	47,0	66,0	86,0	56,7
P.intermedia	33,0	59,0	71,0	46,7
T.forsythensis	53,0	97,0	100	53,3
T.denticola	60,0	82,0	83,0	63,3
C.albicans	10,0	38,0	60,0	21,0

In spite of absolute amount of *T. Denticola* increasing with the progress of disease, its relative part in total bacterial mass in the periodontal pockets increased with increasing of the periodontitis severity. According the qualitative analysis the level of *P. intermedia*and *T. forsythensis*DNA increased significantly (2-3 times) on the initial stage of disease in comparison with healthy periodontium, but was comparatively equal in patients with different periodontitis severity. We have noted the dramatic increase (more than 1000 times) in the level of *P. gingivalis*

in periodontal pockets.

C.albicans amount increased up to 2 x 10^2 in severe CGO cases, which indicates the substantial oral disbiotic process.

3. Conclusions

1. Microbiological monitoring of the periodontal pockets allows to evaluate the condition of the periodontal complex, assess the risk of initiation and progression of the typical and aggressive periodontitis.
2. The real-time PCR is a sufficiently accurate technique for the qualitative and quantitative assessment of the periodontal pathogens in oral cavity.
3. In order to choose the treatment plan and to evaluate the efficacy of the conducted therapy we recommend to investigate the 6 periodontal pathogens in periodontal pockets.

References

[1] *Demchenko T.V. Ivanchenko I.G. Balashov N. V.* (1998) 'Role of a microbic factor in pathogenesis of free radical damages of periodontitis.' // Under the editorship of the prof. of *Kuryakina N.V.* // Actual problems of stomatology: Scientific Conference. - Russian State Medical University, pages 158-160.

[2] *Haffajee A.D., Socransky S.S.* (1994) 'Microbial etiological agents of destructive periodontal diseases.' // Periodontol 2000, vol 5, pages 78-111.

[3] *Holt S.C., Ebersole J.L.* (2005) 'Porphyromonasgingivalis, Treponemadenticola, and Tannerella forsythia: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis.' // Periodontology 2000, vol. 38. pages 72–122.

[4] *Kumar P.S., Griffen A.L., Moeschberger M.L., Leys E.J.* (2005)'Identification of Candidate Periodontal Pathogens and Beneficial Species by Quantitative 16S Clonal Analysis.' // Clinical Microbiology, vol. 43, No. 8, pages 3944–3955.

[5] *Paster B. J., Boches S. K., Galvin J. L, Ericson R. E, Lau C. N, Levanos V. A, Sahasrabudhe A., Dewhirst F. E.* (2001) 'Bacterial diversity in human subgingival plaque'.// J. Bacteriol. 183: pages 3770–3783.

[6] *Simonson L. G., McMahon K. T., Childers D. W., Morton H. E.* (1992) 'Bacterial synergy of Treponemadenticola and Porphyromonasgingivalis in a multinational population.' // Oral MicrobiolImmunol vol.7, pages 111-112.

[7] *Socransky S. S, Haffajee A. D, Dzink J. L, Hillman J. D.* (1988)'Associations between microbial species in subgingival plaque samples.' // Oral MicrobiolImmunol, 3: pages 1-7.

[8] *Takamatsu N., Yano K., He T., Umeda M., Ishikawa I.* (1999) 'Effect of initial periodontal therapy on the frequency of detecting Bacteroidesforsythus, Porphyromonasgingivalis, and Actinobacillusactinomycetemcomitans.' // Periodontol, 70, pages 574-580.