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METAGENOME OF DENTOGINGIVAL SULCUS`S COMMUNITIES BY THE YOUNG PEOPLE WITH INTACT PERIODONTIUM

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Abstract:

Periodontal diseases have a high prevalence all over the world and affect the young contingent. In this regard it is important to study the metagenome of the dentogingival sulcus by young people with intact periodontium, in order to form initial materials for solving various clinical and microbiological problems. The aim of the study was to research the genomic composition of the microbiota of the dentogingival sulcus by the healthy young people with intact periodontium living in the territory of Kazan, Republic of Tatarstan. The study included 11 young people (6 boys and 5 girls) 18-19 years old with intact parodontium. This paper describes a universal algorithm of metagenomic studies and the results of clinical and microbiological studies of the dentogingival sulcus microbiome by the patients without orthodontic and mucogingival pathology. It showed that the relative number of 21 phylotypes at the level of genus and families differed significantly. There were found unique microbial communities that were not found in previously studied metagenomes.

Key words: *Dentistry, Intact Parodontium, Dentogingival Sulcus, Metagenome Of Communities.*

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INTRODUCTION:

Literary reference of the problem. The diagnosis «clinically intact periodontium» appropriate for the patients whose depth of probing of the dentogingival sulcus does not exceed 2.5 mm and there is no supra – and subgingival stone, bleeding of the gums and on the orthopantomogram there is no destruction of bone tissue, traces of osteoporosis, dislocation of the cortical plate [1, 2, 3, 4, 5, 6]. It is also important to note that by a healthy person with an intact pariodontium, the marginal part of the gums is of a pointed shape, during the period of teething the gums become thicker and have rounded edges [1, 7].

To confirm the diagnosis of «intact periodontium» and timely detect inflammatory periodontal diseases it is recommended to test the cellular composition of gingival fluid, which is normally 0.06 mg, and during the day constantly enters the oral cavity with a volume of 0.5-2.4 ml [8, 9]. Gingival fluid is a complex biological environment of the body, which plays an important role in maintaining the normal state of the periodontium. It found glucose, hexosamines, woronowii acid, lactic acid, glycerophospholipid, triacylglycerol. Urea and ammonia that present in the gingival groove fluid maintain the pH of 6.3-7.9. Gingival fluid proteins are involved in the immune response (Ig), contribute to the connection of the epithelium of the gingival groove with the tooth surface, forming an adhesive film. Normally, the activity of enzymes in the gingival fluid is minor, but it changes with the development of inflammation in the periodontium. Proteinases play a special role in the destruction of cellular elements of the periodontium and the development of inflammation. The latter are associated with increased activity of collagenase, which in the physiological state is close to zero. In addition, the gingival fluid has elastase, coming from azurophilic leukocyte granules: cathepsin D, whose activity is much higher in the norm, than in blood plasma. The gingival fluid is also determined by the activity of alkaline and acid phosphatase, hyaluronidase, b-glucuronidase, lysozyme, there are enzymes of glycolysis, tricarboxylic acid cycle (succinate dehydrogenase), aminotransferase, lactate dehydrogenase. The mineral composition of gingival fluid is somewhat different from blood plasma: the amount of Na+ and K+ in it is higher than in gum tissues, but much lower than in plasma. Besides the gingival fluid contains calcium, phosphorus, magnesium, zinc, sulfur, chlorine, fluorine. The protein composition of the waters of the gums and blood plasma is the same. The amount of protein is 60-70 g / l and it does not depend on oral hygiene. Globulin fraction of gingival fluid is represented by enzyme proteins, immunoglobulin G, components of complement system, fibrinolysis, lactoferrin. Among the protein fractions of the gingival fluid, the components of the complement system are also determined, which are important during the reactions developing in inflammation. In gingival fluid there are some amino acids and kinins that affect microcirculation, increasing the permeability of the vascular wall, increase the migration of leukocytes and lymphocytes [9].

Neutrophil leukocytes and epithelial cells are recorded in gingival fluid smears by the persons with intact periodontal [10]. Two stable cell bonds are also observed: lymphocyte-macrophage, lymphocyte-polymorphonuclear leukocyte [11]. The study of the manifestations of cellular immunity in gingival fluid allow to assert that in an the intact periodontium for immune protection on the first place answer T lymphocytes-helper cells type 1 [10].

Gingival fluid is not normally sterile. In intact dentogingival sulcus, the total quantity of microorganisms is small and facultatively anaerobic gram-positive bacteria prevail [7], the proportion of gram-negative bacteria is 10-15% [12]. In people with intact periodontal disease, the frequency of occurrence of five main periodontal pathogens (*Prevotella intermedia, Tannerella forsythensis, Treponema denticola, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*) does not exceed 6.6% [13].

It is known that periodontal diseases have a high prevalence and affect the young contingent, while periodontal tissues are not completely formed [14, 15, 16, 17, 18, 19, 20], in this regard, it is important to study the metagenome of the dentogingival sulcus by the young people with intact periodontium to form initial materials for solving various clinical and microbiological problems.

The aim of the study was research the metagenome of communities of the dentogingival sulcus by healthy young people 18-19 years old with intact periodontium living in the territory of Kazan, the Republic of Tatarstan.

MATERIAL AND METHODS:

The study included 11 healthy young people (6 boys and 5 girls) 18-19 years old with intact periodontal disease. The criteria for choosing this age group were on the one hand – completion of the formation of

permanent teeth, periodontal and reduction of the effect of sympathetic innervation on the growth of jaws (18 years), on the other – the end of the puberty period (19 years) [21]. All participants of the study were of European origin and lived on the territory of Kazan of the Republic of Tatarstan.

Criteria for inclusion in the study group:
1.the age of 18-19;
2.healthy and does not consist on the account in other health care organizations;
3.do not have bad habits-alcohol, tobacco, drug addiction;
4.not pregnant or using hormonal contraception;
5.do not use antibiotics for 3 months;
6.are registered by the periodontist;
7.do not have periodontal pathology;
8.do not have orthodontic pathology.
9.compliance of the periodontal state with clinical and radiological signs of intact periodontal.
Criteria for exclusion from the study: the presence of periodontal disease, patients of another ethnic group.
The Protocol of management of patients diagnosed with intact periodontal disease included:
1.individual oral hygiene training;
2.training in controlled oral hygiene.
Patients signed a voluntary informed consent before the study. Permission of The local ethical Committee of the FGBOU to the Kazan state medical University of the Ministry of health of Russia (extract from Protocol No. 9 of November 22, 2016) was obtained to conduct the study.
By subjects with intact periodontal samples were obtained from the gingival sulcus (five randomly selected teeth). The sampling was carried out after professional oral hygiene and removal of supragingival deposits using sterile curette Gracie (Well–Friedy). A sterile cotton ball with the use of sterile tweezers, placed in the investigated area, without touching the mucous membrane of the mouth and neck of the tooth. The collected samples were placed in 2 ml micro-centrifuge tubes and frozen at -20°C for further DNA isolation and comparative analysis of microbial communities.
Total DNA was extracted and purified from the selected sample using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The total amount of extracted and purified DNA was further measured using The nanodrop ND–2000 spectrophotometer (Wilmington, USA). The resulting total DNA was stored in the freezer at-20C.

Fragments of bacterial 16s rRNA genes were amplified by barcoded primers Bakt_341F ('5-CCTACGGGNGGCWGCAG-3') and Bakt_805R ('5-GACTACHVGGGTATCTAATCC-3') using Fusion High–Fidelity DNA polymerase, in three repeats for each sample. The resulting amplicons for each sample were combined and cleaned using Agencourt AMPure XPbeads (Beckman Coulter, USA). The amount of DNA was determined using Quant–iTds DNAHS Assay Kit. Sequencing was performed using the sequencer ABI 3730 DNA Analyzer (Life Technologies, USA).

The resulting sequences were analyzed using QIIME, Version 1.9.1. Paired readings were combined. Low-quality and chimeric sequences were removed. The remaining sequences were grouped into operational taxonomic units (OTE) at 97% similarity (minimum five sequences for OTE). OTE was administered by the method of open reference. The Kruskal-Wallis test was used to determine the relative abundance of phylotypes between groups. Version R 3.4.1 was used, the value was set to $p<0.05$.

RESULTS AND DISCUSSION:

This paper describes a universal algorithm of metagenomic studies and the results of the first clinical and microbiological study of oral microbiome by the young people 18-19 years old without orthodontic and mucogingival pathology. The study involved 11 people, what exceeds the quantity of all the samples described in the literature, that were studied by this method. In the present study structures of microbial communities were analyzed using sequencing of bacterial 16s rRNA gene fragments (regions V3 and V4). After combining the paired readings, the average length of the obtained sequences was 460 n. p. (n. p. is the length of DNA fragments in nucleotide pairs). On average, there were 34,600 sequences per sample.
It should be noted that in all cases it is a question of determining the composition of the metagenome of the dentogingival sulcus by the results of DNA sequencing from samples, which roughly corresponds to the impression of the periodontal microbiota by the healthy individuals. The inhabitants of the dentogingival sulcus closely interact with each other, so talking about the role of microbiota will be correct to consider its full set.

Any community is not just the sum of its constituent species, but also a set of interactions between them. One of the important properties of the community,

which reflects its complexity and structure, is the diversity. Species diversity reflects the complexity of the structure and structure of the community. Figure 1 shows the alpha diversity of the samples-the diversity within the communities, the so-called species abundance.

The use of alpha–diversity indices made it possible to indirectly determine their status (table 1). There were identified 183 phylotypes at the level of genus relating to 17 phylums which is a synonym of type in taxonomy. Table 1 shows the 46 most numerous phylotypes at the level of genus.

To identify differences in the relative number of phylotypes at the level of genus and families between samples, the Kruskal–Wallis test was used to determine the equality of the medians of several samples (this criterion is a multidimensional generalization of the Wilcoxon – Mann – Whitney test).

Because the most of the detected microorganisms accounted for a very low percentage of the total, many were not present in all samples. Most likely, these were minor microorganisms that do not make a significant contribution to the processes occurring in the studied biotopes, and it makes no sense to list them all.

If we talk about statistical significance, the statistical program used counted about 46 phylotypes, the difference was formally statistically significant, but half of these phylotypes are present in very low quantities – about 0.001%, what shows the relative abundance of species in each study group, which was expressed as a median value and the spread from the maximum to the minimum group (for example, 0.00 (0.00 to 0.33).

The majority of samples were dominated by representatives of the genus *Streptococcu*s. The proportion of *Streptococcus* was significantly dominant in patient microflora samples (31.73 (6.11 to 50.30). The second of the predominant groups in intact periodontium was the genus Neisseria (8.50 (0.03 to 18.18). In addition, patients with intact periodontal disease were associated with members of the family *Micrococcacea* and genus *Rothia* – 5.35 (0.13 to 13.30). Interestingly, the genus *Actinomyces* 2.46 (0.27 to 16.13) and *Veillonella* 3.65 (0.36 to 10.19) and *Fusobacterium* (5.16 (0.39 to 14.97) were identified by the significant quantity of young people in this group. Besides it is known that the genus *Fusobacterium*, which includes renowned pathogens, have the ability to intracellular parasitism and in some cases, representatives of this genus make a significant contribution to the development of inflammatory periodontal diseases.

Members of the genus *Selenomonas*, *Corynebacterium*, *Campylobacte*r were also present in the dentogingival sulcus, but in a significantly lower quantity.

Also there were found unique microbial communities that weren`t found in previously studied metagenomes samples – uncultivated representatives of the families *Gemellaceae, TM7-3, Lachnospiraceae, Weeksellaceae, Mogibacteriaceae, Bacteroidales, Tissierellaceae, Rs-045.*

CONCLUSION:

There was analyzed the microbiome of periodontal spaces (periodontal sulcus and periodontal pocket) by the patients 18-19 years old with inflammatory periodontal diseases and without orthodontic and mucogingival pathology. The variety of bacteria was significantly high. It shows that the relative number of 21 phylotypes at the level of genus and families differed significantly. There were found unique microbial communities that were not found in previously studied metagenomes and the established level can be used as starting materials for solving various clinical and microbiological problems. At the same time, a characteristic like a quantity of genes in the metagenome may eventually become a diagnostic tool for the detection of inflammatory periodontal diseases. And the presence of samples with abnormally high DNA content can serve as an indirect sign of inflammatory processes or excessive desquamation of the epithelium. In addition to assessing the quality of experimental procedures, the result of this filtration can serve as a primary marker of possible pathology.

FINDINGS:

The study of the genomic composition of the microbiota of the dentogingival sulcus showed significant differences in 21 phylotypes at the level of genus and families. There were found unique microbial communities that were not found in previously studied metagenomes and the established level can be used as starting materials for solving various clinical and microbiological problems.

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The commune, 9, phone: (843) 2930280 (head proteogenomic direction, the Director of the Interdisciplinary centre for proteomic research, Chernov V. M., +7 (917) 926-29-91, chernov@kibb.knc.ru) and expressed appreciation to his colleagues for help.

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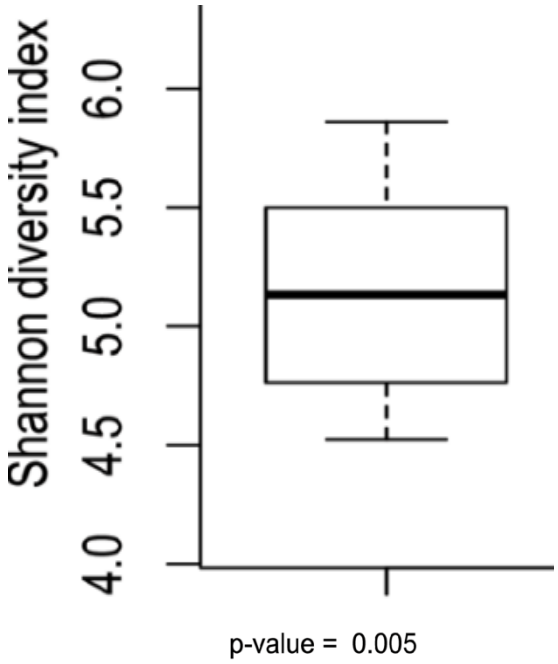

Figure 1. Alpha-diversity of microbial communities of dentogingival sulcus in young people with intact periodontium

Table 1. Relative abundance of species / phylotypes identified in the dentogingival sulcus in young people with intact periodontal disease

Species / phylotypes	Median value and variation
Streptococcus	31.73 (6.11 to 50.30)
Neisseria	8.50 (0.03 to 18.18)
Rothia	5.35 (0.13 to 13.30)
Fusobacterium	5.16 (0.39 to 14.97)
Veillonella	3.65 (0.36 to 10.19)
Granulicatella	3.46 (0.07 to 5.49)
Prevotella	3.27 (0.41 to 11.23)
Actinomyces	2.46 (0.27 to 16.13)
Leptotrichia	1.87 (0.14 to 16.97)
Haemophilus	1.51 (0.00 to 14.65)
unclassified *Gemellaceae*	1.50 (0.05 to 6.38)
Capnocytophaga	0.85 (0.10 to 4.23)
Porphyromonas	0.68 (0.02 to 9.74)
Escherichia	0.63 (0.04 to 26. 51)
unclassified *TM7-3*	0.41 (0.08 to 21.51)
unclassified *Lachnospiraceae*	0.38 (0.00 to 6.48)
Oribacterium	0.25 (0.00 to 2.14)
Corynebacterium	0.21 (0.02 to 1.22)
unclassified *Weeksellaceae*	0.18 (0.01 to 0.71)
Campylobacter	0.15 (0.08 to 5.76)
Lautropia	0.15 (0.01 to 2.33)
Parvimonas	0.12 (0.00 to 0.60)
Comamonas	0.10 (0.00 to 4.56)
Selenomonas	0.10 (0.00 to 1.85)
Aggregatibacter	0.08 (0.01 to 3.18)
Eikenella	0.08 (0.01 to 0.64)
Atopobium	0.08 (0.00 to 2.60)
Tannerella	0.07 (0.00 to 1.23)
Bulleidia	0.07 (0.00 to 0.64)
unclassified *Lachnospiraceae*	0.06 (0.01 to 2.45)
unclassified *Mogibacteriaceae*	0.06 (0.00 to 0.76)
Paludibacter	0.05 (0.00 to 0.73)
Abiotrophia	0.05 (0.00 to 0.55)
Actinobacillus	0.04 (0.00 to 1.91)
Treponema	0.04 (0.00 to 0.54)
Dialister	0.03 (0.01 to 0.74)
Megasphaera	0.03 (0.00 to 0.75)
Peptostreptococcus	0.01 (0.00 to 0.65)
unclassified *Dethiosulfovibrionaceae*	0.01 (0.00 to 0.58)
unclassified *Bacteroidales*	0.01 (0.00 to 0.05)
Halomonas	0.00 (0.00 to 3.60)
unclassified *Tissierellaceae*	0.00 (0.00 to 0.33)
Filifactor	0.00 (0.00 to 0.32)
Schwartzia	0.00 (0.00 to 0.16)
unclassified *Leptotrichiaceae*	0.00 (0.00 to 0.12)
unclassified *Rs-045*	0.00 (0.00 to 0.01)