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Research Article

Quantitative Analysis of Microbiota in Patients with Orthopedic Structures on Dental Implants Using the Real-Time PCR Method

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ABSTRACT
Microbiological research was carried out by PCR method in 42 persons aged 25-60 years (men - 24 and women - 18), installed and Osseo integrated DIO dental implants, which were prototyped by metal-ceramic crowns, with temporary fixation on Tempo Bond NE. After 20 days, a follow-up visit was scheduled.
All the examined patients were taken biological substrate of gingival fluid from the para-implant furrow using paper endodontic pins (№ 25). Exposure of two pins was made within 30 seconds with the help of tweezers between the peri-implant cuff and the crown, biological substrate of gum liquid was taken from the parai-implant furrow.
The biological substrate was transported and tested according to the manufacturer's recommendations.
When studying the content in patients with orthopedic constructions on dental implants by PCR method we observed high frequency of occurrence of Streptococcus sobrinus (69,3%), Streptococcus sanguis (48,6%) and Porphyromonas gingivalis (41,9).
Relatively similar data on the content of these microorganisms were obtained in the molecular genetic study of Streptococcus oralis samples (22.7%).
Obviously, it is the combined effect of the most frequently diagnosed pathogens and peculiarities of interaction of anaerobic agents of parasitocenosis that can determine the nature of inflammatory and destructive process in the peri-implant zone.

Keywords: PCR, pathogenic, opportunistic pathogens, internal interface, paraimplant area, implant, abutment.

INTRODUCTION

Dental implantation continues to be one of the most important directions among the priority problems of dentistry in the modern world. Application of dental implants solves a significant part of problems in case of partial and complete absence of teeth, plays a decisive role in restoring the function of chewing, helps to correct and improve the aesthetics of the tooth row, smile and face in general.
The experience of dental implants installation continues to accumulate in all its aspects [2,3].
One of the conditions for the smooth postoperative flow is the decrease of the microorganisms' level in the implant bed area [1,4,9].
In spite of the fact that implantation in recent years has a high level of success in the early postoperative period, in the scientific literature there is more and more information about the risk of distant complications connected first of all with the development of inflammation of tissues surrounding the Osseo integrated implant [4,7,9,12].
The most likely cause of development in the tissues surrounding a functioning implant may be penetration of the oral infection into the area of implant-bone contact. The microbial composition in peri-implant is now known and represents a large variety of aerobic and anaerobic microorganisms. The researchers attach special importance to Prevotella intermedia, Porphyromonas gingivalis, Actinobacillum actinomycetam commitans, Bacteroides forsithus, Treponema denticola [8,10,11]. The inflammatory process of tissues in the peri-implant zone is the main reason of bone tissue destruction and resorption in the implant area.
The source of bacterial flora may be gaps and hollow spaces in the inner surface (interface) of the implant abutment, which will act as a bacterial reservoir. Since usually used implants consist of two parts - the implant and the abutment - there is a connection place between them, which is the inner surface (interface) of the implant. The technologically acceptable gap is from 2 to 5 μm, which is quite enough for penetration of typical representatives of pathogenic microflora of the oral cavity with comparable sizes - from 0.5 to 2.0 μm.

OBJECTIVE OF THE STUDY

To detect pathogenic microorganisms in patients with orthopedic constructions on dental implants by PCR method.

RESEARCH MATERIAL AND METHODS

In order to solve the stated goal we carried out microbiological research in 42 people aged 25-60 years (men - 24 and women - 18), who were undergoing treatment at the Department of Surgical Dentistry and dental implantology of TGSI. This system has an implant-abutment connection by the type of hexagonal connection using a fixation screw. All patients had orthopedic constructions (metal-ceramic crowns, with temporary fixation on Tempo Bond NE) installed. After 20 days, a follow-up visit was scheduled. The oral consent of the patients was obtained, to undergo the examination.

The fence of material for bacteriological examination was carried out in the area of implant gum furrow using paper endodontic pins (№25). Exposure of two pins was made within 30 seconds with the help of tweezers between the peri-implant cuff and the crown, biological substrate of gingival fluid was taken from the parai-implant furrow. It was then placed in a sterile Eppendorf plastic tube (1.5 ml) containing 1 ml of physiological solution. The samples were stored and transported to the laboratory at +4°C for 2 hours. Sample batches were transported to the laboratory in thermal containers with the refrigerant.

For DNA testing of the samples with the reagent set "Ampley Prime® Florocenose-Aeroba" and the reagent set for quantitative determination of human DNA recommended by the manufacturer were used, and the results were calculated normalized values of microorganism DNA concentration reflecting the number of microorganism cells relative to human mucous membrane cells.

At the stage of amplification with detection, reagent tubes, DNA samples and control samples were injected using disposable filter tips. When preparing the amplification tubes the total volume of reaction mixture was - 25 μl, including the volume of DNA sample - 10 μl.

For one reaction 10 μl of PCR mixture of Aeroba Florocoenosis and 5 μl of PCR buffer-H are required. The mixture was prepared for the total number of tested and control samples plus a stock for several reactions.

The contents of the tubes were mixed with PCR-mixture of Florocenosis-Aeroba, PCR-buffer-H, deposited droplets on the vortex. In a separate tube prepared a reaction mixture, mixed the necessary amount of PCR-mixture of Florocenose-Aeroba and PCR-buffer-H, precipitated the drops on Vortex. The necessary amount of tubes was taken for DNA amplification of the tested and control samples. Each tube was filled with 15 μl of the prepared reaction mixture and 10 μl of DNA samples obtained as a result of extraction from the investigated samples.

Detection of the fluorescent signal is assigned through the channels for fluorophores FAM, JOE, ROX and Cy5. If other tests are performed at the same time, detection is also assigned to other used channels.

Tubes are placed in the cells of the reaction module of the device and start the amplification program with fluorescent signal detection.

On the basis of the obtained threshold cycle (ST) values and on the basis of the given concentration values for DNA calibrators K1 and K2, the automatic construction of the calibration straight line and calculation of the Genome Equivalents (GE) values of streptococcal DNA in 1 ml of the investigated and control samples are performed.

The obtained results of index evaluation were processed in accordance with the principles of medical statistics using the package of programs "Excel-7", "Statistica 5.0" with the use of non-parametric methods of quantitative characteristics analysis.

RESEARCH RESULTS AND DISCUSSION

The extraction of the internal space of a dental implant using sterile paper endodontic pins (size 25) is the most optimal way to take the material for molecular genetic research, due to the excellent absorption capacity of the pins, the possibility of taking the clinical material of a certain volume and the elimination of trauma of periimplant tissues.

Qualitative evaluation of the internal space content of the dental implant by microorganisms in clinical samples. While studying the content in patients with orthopedic constructions on the dental implants by PCR method we observed high frequency of occurrence of Streptococcus sobrinus (69,3%), Streptococcus sanguis (48,6%) and Porphyromonas gingivalis (41,9%) species (figure 1).

Relatively similar data on the content of these microorganisms were obtained in molecular genetic studies of Streptococcus oralis samples (22.7%).

The above data indicate the predominance of facultative anaerobic and bonded anaerobic flora due to the lack of free access to oxygen in the studied space.

The majority of species of the detected microorganisms are conditionally pathogenic, some - saprophytes. Such a high proportion of anaerobic agents and their variety make it difficult to identify a leading pathogen that could be the "leader" of the infectious-inflammatory process. Obviously, it is the combined effect of the most frequently diagnosed pathogens and the interaction of anaerobic agents of parasitocinosis that can determine the nature of the inflammatory and destructive process in the peri-implant zone.

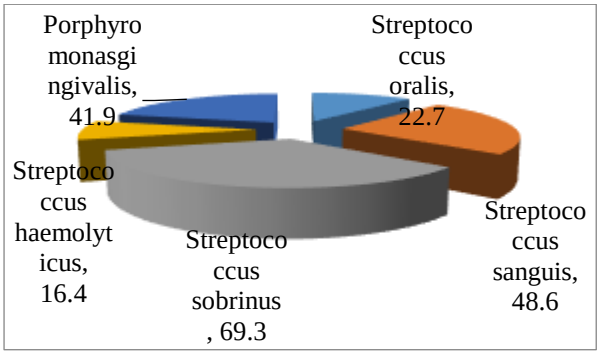

Fig.1. Frequency of detecting pathogenic and conditionally pathogenic bacteria of the dental implant inner space by PCR method

If we take into account the fact that it is the foci of the listed chronic infection that are often the basis of periodontal disease, we can assume that if bacteria can expand from the implant interface into the oral cavity, inflammatory processes can occur in the peri-implant tissues, which subsequently lead to instability of the implant.

CONCLUSION.

Thus, we carried out work on standardization of detection and quantitative determination of pathogenic and conditionally pathogenic bacteria of the internal space of dental implant microorganisms in the clinical material by PCR method in real time. The following conclusions can be drawn:

1. According to the conducted research it is possible to draw a conclusion that the presence of the fixture and inner cavity superstructure in the area of the dental implant articulation leads to the fact that there is a migration of bacteria and products of their vital functions from the external and internal interface of the dental implant, regardless of the linear sizes of these spaces.

2. A considerable number of microorganisms from Gram-positive coccuses to Gram-negative sticks turned out to be able to penetrate through the gap between the implant and the abutment.

3) It is possible to assume that the gap in the area of the implant and abutment articulation influences the processes of osseointegration and inflammatory reaction in the peri-implant zone tissues, but more detailed and profound research is to be carried out on the interrelation between the microleakage volume in the articulation interface and bone tissue loss.

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