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FEATURES OF THE COURSE OF PERIODONTAL TISSUE DISEASES IN CHILDREN WITH ADENOVIRUS INFECTION, THE USE OF EXAMINATION METHODS.

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Abstract

Modern methods of molecular diagnostics currently used make it possible to successfully study the oral microbiome, quickly detect periodontal pathogens present in diagnostic biomaterial, even in small quantities, and identify clinically significant uncultivated and difficult-to-cultivate types of microorganisms. Considering the above, today a combination of different methods for each specific case is the most optimal.

Keywords

Teeth, periodontitis, periodontitis, inflammation, adenovirus, bite, gingivitis.

Due to all the above advantages, in the modern world, the PCR method is considered the gold standard for identifying etiological factors involved in the progression of periodontal diseases [31, 32]. Isothermal loop amplification (LAMP). LAMP is a common method for quick and sensitive diagnosis. This method can also be considered the most promising for analysis in conditions where time and resources are limited, which is ideal for determining the microbiota of periodontal pockets and determining effective treatment in dental clinics. LAMP uses DNA polymerases characterized by chain-displacing activity and 4-6 primers to provide a more specific reaction[3].

Eventually, as a result, specific structures are formed from repeated inverted sequences of the original target DNA, linked together by loops of single-stranded DNA. The LAMP method effectively increases the amount of DNA by 109-1010

times in 15-60 minutes, thanks to the use of DNA polymerases with chain-displacing activity, which ensures high amplification efficiency. In addition, the LAMP method can be used for RNA amplification if reverse transcriptase is added to the reaction mixture[5].

The LAMP method on a microchip takes from 15 minutes to an hour, which is less than is usually required for PCR. Microsystems for both methods are sensitive and specific, but LAMP is preferable due to the isothermal reaction mode, which makes it simpler and more accessible. In addition, the variety of visual detection methods for LAMP products allows you to choose options that do not require the use of special equipment to detect positive and negative results. These systems are often used for preliminary testing or rapid monitoring. If quantitative LAMP analysis is required, standard dilutions or internal controls are required[1,6].

However, the complexity of multiplex analysis is a limitation of the LAMP method, but due to the immobilization of primers in the microstructures of the chip, amplification and detection of different DNA fragments simultaneously in different chambers can be carried out using the same intercalating dye [33]. Currently, various commercial kits exist for the identification of Escherichia coli and Listeria monocytogenes by the LAMP method [34]. This method is also used to identify DNA viruses such as human herpes simplex virus (HSV), adenoviruses and others, to detect parasites, such as toxoplasma. It is interesting to use this method for the destruction of genetically modified products by combining LAMP with immunochromatography [8].

The advantages of loop isothermal amplification include the ability to identify individual bacterial strains (from DNA or from whole cells) in a highly specific and rapid way through visual interpretation of the results. All this makes it possible to use the LAMP method in dental clinics to simplify and speed up the diagnosis of periodontal diseases. Sequencing of the 16S rRNA gene. Sequencing of the conserved 16S rRNA gene is another method of molecular diagnosis of periodontitis. There are unique differences between the sequences of this gene in different bacteria, which make it possible to identify the analyzed bacteria to a genus or even to a species [3].

Sequencing at the initial stages is carried out using PCR with primers matched to the 16S rRNA gene. The PCR product is then sequenced and the resulting sequences are compared with databases of known bacterial species [22]. The first global database containing information about oral microorganisms was the HOMD (Human Oral Microbiome Database). It contains data on almost 700 species of bacteria living in the human oral cavity. About 49% of them have an official name,

17% have no names, and 34% are considered uncultivated phylotypes, i.e. taxonomic units of various ranks: strains, species, genera (www. homd.org).

HOMD tools allow you to compare the sequence of analyzed bacteria with the phenotypic, phylogenetic and clinical information available in the database. It is assumed that if the sequence of the 16S rRNA gene coincides by at least 97% with the known sequence from the database, then the studied bacteria can be attributed to the genus, and if the coincidence is 99%, then it can be attributed to a specific species [23]. Another advantage of 16S rRNA sequencing is the selection of highly specific primers for certain groups or strains of bacteria and the possibility of their amplification from material samples. This makes it possible to diagnose infections caused by uncultivated bacteria[5,8].

The disadvantage of this method is its low efficiency in separating closely related and highly recombined species, for example, species from the genus Neisseria and some species of the genus Streptococcus. Despite this, sequencing of the 16S rRNA gene has revealed more than 300 bacterial species that had not previously been identified by standard cultivation methods [3]. The frequency of A. actinomycetemcomitans, P. gingivalis, T. forsythia, and T. denticola was determined using 16S rRNA sequencing [5].

Bacteria that cause periodontitis have been found in other parts of the human body, where these bacteria can be found in focal infections [36]. The method of sequencing the 16S rRNA encoding gene can also be useful in the diagnosis of endoparodontal infections, as it determines the bacterial composition in the lesion and allows determining the source of infection [7].

Thanks to sequencing, it is possible to study the composition of the entire oral microbiome with the determination of changes under the influence of various factors. For example, to compare and study the microbiome of the subgingival plaque of smokers and non-smokers associated with inflammation around dental implants [8].

Next Generation sequencing (NGS). In recent years, there has been a significant development in DNA sequencing technologies. Mass parallel or deep sequencing are terms referring to the DNA sequencing technology that has revolutionized genomic research. Using NGS, the entire human genome can be sequenced in one day. Next-generation sequencing has found applications in identifying and understanding the biodiversity of viral genomes, including influenza, HIV, and viral hepatitis B [9].

This method was used to evaluate changes in the composition of the subgingival microbiome in patients with periodontitis after treatment, and was also compared with the microbiome of periodontal pockets in smokers and non-

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smokers [4]. NGS sequencing is an excellent tool for studying the diversity of biofilms found in the human oral cavity [8].

This method is successfully used in the molecular diagnosis of periodontal inflammation, but standardization of the method is required [12]. Microchips using the hybridization method. Microarrays using the hybridization method are used to identify microorganisms and determine gene expression. They consist of single-stranded probes bonded covalently to the glass or nylon surfaces of the microcircuit. Probes in the form of single-stranded DNA fragments with a known sequence, PCR products or oligonucleotides are used to detect specific fragments of nucleic acids. The probes are designed for hybridization with specific RNA or DNA sequences from a test sample of biological material[3].

The sequence of probes is most often selected from the GeneBank or UniGene databases [5].

All commercially available microchip kits have a single mechanism of action. After applying the sample to the surface of the chip, the desired single-stranded fragment of nucleic acid is hybridized with a complementary probe. Double-stranded fragments are formed, which are detected by fluorescent, chemiluminescent or mass spectrometric methods. The intensity of the signal received from the analyzed sample makes it possible to determine the amount of bound nucleic acid, and thus estimate the number of microorganisms or the level of gene expression in the tested material [5]. In this way, it is possible to identify agents related to the virulence of microorganisms, for example, antibiotic resistance genes. DNA microarrays have unlimited capabilities for detecting different DNA sequences. They can contain from hundreds to thousands of probes on their surface, and high-density microchips contain from thousands to millions of molecular probes [8].

Commercial DNA chips identify biofilm microorganisms in periodontitis. The microchip for clinical periodontal diagnostics ParoCheck® allows detecting 10 types of associated bacteria with periodontitis [4].

Conclusion. Thus, biofilms in the oral cavity represent complex interactions between communities of microorganisms, and their composition is of great importance for the course of periodontal diseases, which indicates the need for the most sensitive, specific, and rapid diagnostic methods. Modern methods of molecular diagnostics currently used make it possible to successfully study the oral microbiome, quickly detect periodontal pathogens present in diagnostic biomaterial, even in small quantities, and identify clinically significant uncultivated and difficult-to-cultivate types of microorganisms. Considering the above, today a combination of different methods for each specific case is the most optimal. This

approach in the etiological diagnosis of periodontitis patients makes it possible to successfully select the most effective treatment methods, but additional research is needed to improve, standardize and reduce the cost of the described methods.

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