Microbiological Aids in Periodontics

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Abstract

New approaches to periodontal diagnosis, including advanced microbiologic, biochemical, and genetic tests, have been shown to provide the clinician with the information not available by traditional means. The purpose of a diagnostic test is to confirm, exclude, classify, or monitor disease to guide treatment. Their clinical value depends on whether the information they provide leads to improved patient outcomes. This can be assessed by randomized trials, which compare patient outcomes from the new diagnostic test versus the old test strategy. So, the current paper aims to focus on the practical utility of this rapidly emerging plethora of periodontal microbiological diagnostic tools, emphasizing the critical issues surrounding the clinical application of microbiologic investigations, employed for periodontal diagnosis.

Keywords: *Microbiologic investigations; Periodontal diagnosis; Clinical application.*

Introduction

A clinical diagnosis of periodontal disease does not indicate the prognosis, causative agents, progress and prognosis of the disease. For several decades, the microbiology of periodontal disease has been the focus of intense investigation. This focus is justifiable since bacteria (*A.actinomycetemcomitans, P. gingivalis, P. intermedia*, etc) are the etiological agents of periodontal diseases. Microbiological tests are used not only to identify the putative pathogens and indicators of disease initiation and progression but also utilized to observethe treatment of periodontal disease directed at the suppression or elimination of periodontal pathogenic microorganisms.[1]

Microbiological Aids:

1. **Direct Microscopy:** Dark-field or phase- contrast microscopy is the oldest method of microscopy and it is used to identify spirochetes and other motile organisms in plaque sample.[1] However, most of the putative periodontal pathogens like *Aa*, *Pg, Tf;* which are nonmotile and therefore this method cannot identify these species. Therefore this technique is not ideal for the diagnosis of periodontal diseases. This method can use to educate and motivate the patient.[2]
2. **Bacterial Culturing:** Bacterial culturing is still considered as a reference method (gold standard) against the newer techniques which are compared to establish their efficacy.[2] Generally samples are cultivated under anaerobic conditions; due to the majority of periodontal pathogens are anaerobes in nature.[3] Several systems perhaps used to create an anaerobiotic atmosphere are;

- Bio Bags
- Pre Reduced Anaerobically Sterilized Media (PRAS)
- Anaerobic chambers and anaerobic jars

Among the above, anaerobic jars and anaerobic chambers are most frequently used. These are used with a coalesce of basal and selective media for isolation of periodontal pathogens.[3]

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3. **Immunodiagnostic Method:** Immunologic assays recognize bacterial antigens to detect target micro-organisms. This reaction can be revealed by various procedures like; Immunofluorescent assays (IFA), Enzyme-linked immunosorbent assays (ELISA), Flow cytometry, Latex agglutination, Membrane assays.[2]

Immunofluorescent Assay: It is of two types; Direct immunofluorescent assay and Indirect immunofluorescent assay. Indirect immunofluorescent assay,the antigen is reacted directly with a fluorescein-conjugated antibody. In indirect immunofluorescent assay, the secondary fluorescein-conjugated antibody reacted with primary antigen-antibody complex.[4]

Flow Cytometry: It is also known as Cytofluorography. It allows detection of single or multiple microbes in plaque samples, based on their atypical cytometric parameters. The bacterial cell labeled with a second fluorescein-conjugated antibody and also with species-specific and after that these are undergoing for separation of bacterial cells into a single cell suspension by using flow cytometer. The implementation of flow cytometry in clinical microbiology is not yet widespread due to lack of approach or due to lack of knowledge.[5,2]

Enzyme-linked immunosorbent assay (ELISA): ELISA is the widely accepted, simple but sensitive method.[6] In this method, for labeling purposes, an enzymatically derived color reaction is used. The strength of color is directly proportional to the concentration of antigen. This method is previously used to identify the serum antibodies to periodontal pathogens but now a dayby the help of specific monoclonal antibodies, it has been also used for subgingival samples, to express the amount of specific pathogens present in this sample.[2]

4. **Enzymatic Method:**

BANA Test: The"red complex" bacteria like Porphyromonasgingivalis, Treponema denticola, Tannerella forsythia are colonizing the surfaces at or below gingival and leads to the destruction of periodontal ligament and alveolar bone. This destruction of PDL and alveolar bone causes loss of attachment to the tooth.[7] The above-mentioned species have trypsin-like enzymes and also share a common enzymatic profile.[2] N-benzoyl-DL-arginine-2-naphthylamide (BANA enzymatic test)is a rapid and reliable chair-side diagnostic test, which can give information about the bacteria by hydrolyzing the trypsin-like enzyme.[7]

During hydrolysis, it releases chromophore naphthylamide. A drop of garnet is added to the solution and the solution turns into orange-red. Positive BANA findings indicate Td, Pg, or both are present at sampled sites.[2] In this test, there is always a lack of quantitative data and they cannot identify the pathogens which do not produce trypsin-like enzyme.[6]

5. **Diagnostic Assays Based on Molecular Biology Techniques:**

DNA Probes: These DNA Probes are single-stranded pieces of nucleic acid. Under suitable conditions (pH, temperature and ionic strength), an enzyme which is labeled on DNA probesis help in hybridization with complementary single-stranded DNA or RNA and then detect micro-organisms. The principle of working of DNA probes is sequencing the DNA base.[8] Commercially available DNA probes are DMDx, Omnigene.[2]

Checkerboard DNA-DNA Hybridization Technology: In the year 1994, according to Socransky et al, DNA checkerboard is a method that gives a synchronous and significant analysis of up to 28 plaque samples against 40 microbial species.[9] The assays use DNA probes, which are labeled by digoxigenin and are whole genomic. In this technology, for identification of cells of each microbial species, the DNA probes are used and these are usually modified to allow identification of cells of each species.[2] This technique is used for ecologic studies and also for epidemiological research, because they do not need bacterial viability.[6] It does not provide an estimate of the total bacterial mass in the specific site and also this technology needs sophisticated lab equipment, which are demerits of this technique.[6]

Polymerase chain reaction (PCR): In the year 1983, PCR was introduced by Mullis and he won Nobel Prize for this discovery. The development of polymerase chain reaction is beneficial in genetic analysis for the diagnosis of genetic diseases. It has appeared as a very powerful tool, which allows development, knowledge about transcription of RNA and genes. The PCR test was able to identify periodontal pathogens (Porphyromonasgingivalis) more often than cultivation. Various category of PCR are: Nested polymerase chain reaction, Quantitative polymerase chain reaction,multiplex polymerase chain reaction Real-time polymerase chain reaction, Allele-specific polymerase chain reaction, and Colony polymerase chain reaction.[10]

Conclusion

There are many potential markers for periodontal disease activity and progression. Reinforcement of the identification of periodontal disease can be done by microbiological aids. It also can be utilized to guide the treatment of periodontal disease, so that the suppression and eradication of causative organisms can be done. In all microbiological tests, ranging from interacting bacteria thatare capable of causing periodontal diseases is difficult and the combination may differ between individuals. After all these years of intensive research, we still lack a proven diagnostic test that demonstrates a high anticipating value forthe advancement of the disease, has a proven effect on disease prevalence and incidence, and safe, simple and also cost-effective.

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