Diagnostic Methods for Evaluation of Microbial Flora in Periodontitis

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Summary

Although over 300 bacterial species make up the oral flora, it is thought that only a few, either alone or in combination, initiate the progression of periodontitis. For over 20 years, culture techniques have been the primary method of identifying and studying putative pathogens. Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus *and* Treponema denticola *are considered bacterial pathogens. Culture techniques enable versatility in characterizing the subgingival flora, allow for speciation and antibiotic susceptibility testing. Selective culturing involves the use of media restrictive to certain microorganisms, while nonselective media provides maximal growth and captures a predominant cultivable flora. In addition to technical problems, cultivating microorganisms can be both time consuming and costly. Molecular diagnostic techniques, DNA probes and polymerase chain reaction are especially useful in detecting those bacteria and viruses that cannot be cultivated* in vitro *or are not sensitive to current cultivating techniques. Sensitivity and specificity is optimal according to the great number of bacteria present in plaque samples.*

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Introduction

With the application of contemporary microbiological methods significant improvements have been made in the field of periodontal diagnostics. Thus diagnostic tests have an increasing role in prompt clinical diagnostics, in the determination of optimal therapeutic procedures and increased therapeutic success.

These tests are based on the detection of bacterial plaque, either by identifying the inflammatory mediators, decomposed tissue products or detection of bacterial antigens. According to Socransky (1) four factors need to be present for the occurrence and

development of a periodontal process: tendency of the host, due to weakened defence mechanisms, suitable local environment, increased number of pathogenic bacteria and a reduced number of non-pathogenic factors which inhibit the process. Today, periodontal disease is considered primarily a polybacterial manifestation connected with certain bacterial pathogens (1, 2).

In spite of the presence of a large number of microorganisms identified from a periodontal pocket, the following are considered periodontal pathogens: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Bacteroides forythus*, *Prevotella intermedia*, *Eicenella corrodens*, *Fusobacterium nucleatum* and *Treponema denticola*.

According to the latest findings of Page (3) the role of bacteria is decisive. However, host factors determine the presence, progression and outcome of the disease. Thus, epidemiological and clinical investigations of periodontitis prompted clinicians and investigators to link periodontitis with the characteristics of risk persons for periodontal disease. Namely, the disease manifests in phases of activity and remission, so that the majority of patients do not have a frequent active phase of the disease (this can be occasional and irregular). However, in a small percentage of periodontitis disease progression is frequent and rapid, and reaction to therapeutic measures is unsatisfactory. As we cannot predict active from inactive phases of the disease the question arises of differentiating persons with high risk from persons with low risk of periodontal disease.

According to Page (4) the latest indicators in periodontology are directed to explaining the correlation between oral health and systemic diseases. Also, progression of periodontal disease occurs via mechanisms which may be caused by a firm link between oral and systemic diseases. This may include: activation of the host response, transition of inflammatory mediators into the circulation and an increase in gram-negative bacteria and their components in subgingival biofilm. Microbal colonisation, proliferation and plaque formation on the surface of the tooth, which is covered by different types of periodontal tissue, leads to the question of whether periodontitis is a unique entity. Lindhe (5) considers periodontitis to be a unique disease entity because of its anatomic features.

More recently the possibility of herpes virus participation has been considered in the etiology and pathogenesis of some aggressive forms of periodontitis, which is known as herpes virus associated with periodontal disease. Epstein Barr virus tip 1 (EBV-1) attacks periodontal B-lymphocytes and human cytomegalovirus (HCMV), periodontal monocytes/macrophages and T-lymphocytes. In periodontal lesions, associated with herpes virus infection, there is often a raised level of periodontal pathogenic bacteria (6).

Pathogenically, a periodontal pathological process may be a consequence of either primary virus infection and recurrence or a virus mediated by damaged host defences.

Tests for evaluation of periodontal pathogens

Contemporary knowledge of the etiopathogenesis (7) of periodontal diseases has helped better understanding of the occurrence and course of periodontal diseases, detection of the active phases of the disease and recognition of the factors which may cause damage to the defensive mechanisms of the host. Furthermore, knowledge of the disease activity and mechanisms of pathogeneses will enable practitioners to rapidly and effectively treat periodontal disease.

The connection between periodontal pathogens and disease activity serves as the basis for application of microbiological tests in periodontology. Various types of microbiological tests can be used for: evaluation of etiological factors, evaluation of disease activity, determination of treatment effects and monitoring the recall interval.

Numerous tests can be used for detection of subgingival microorganisms, such as phase-contrast microscopy, dark-field microscopy, bacterial culture, immunological tests, nucleic acid tests, enzyme tests and polymerase chain reaction (PCR).

The quality of different microbiological tests are measured by evaluation of standard criteria, such as sensitivity, specificity, accuracy, most often in comparison with bacterial culture which serves as a "gold standard" (7).

Phase-contrast microscopy and dark-field microscopy

The size, shape and motility of bacteria are evaluated by this method. Samples of plaque connected with healthy condition, characterised by a small number of mainly immotile cocci and samples of plaque associated with disease, characterised by a large number of different bacterial morphotypes are differentiated. While clinical examination is a direct measure of gingival inflammation, dark-field and phase-contrast microscopy can provide information which is not clinically visible. The clinical efficacy of the therapeutic procedure and effectiveness of oral hygiene can thus be shown in the change from flora connected with disease to flora connected with health (8).

Bacterial culture

Microorganisms which are today considered responsible for periodontal disease are mainly anaerobic bacteria. Cultivation of anaerobic bacteria is relatively slow, complicated and expensive. Sensitivity tests are particularly problematic, i.e. determination of resistance, particularly in the case of therapeutically resistant periodontitis. The basic mechanism of resistance to antibiotics is the production of beta-lactamase. Approximately 70% of the species *Prevotella spp* and *Porphyromonas spp*. are sensitive to penicillin, ampicillin and a group of anti-pseudomonas penicillin (tikarcilin, mezlocilin, piperacilin) but only 5-20% in the group of *Bacteroides fragilis*. During the last ten years some species have appeared which are resistant to metronidazol and amoxillin-klavulanat (9).

Bacterial culture is used as a golden standard, according to which other methods are evaluated. Bacteria from periodontal pockets however is difficult to cultivate, which can result in a false negative test in comparison with microbiological tests which do not require cultivated or live bacteria to detect the species concerned. Culture tests determine the bacterial cells or units which form colonies, while immunological tests and probes of nucleic acids measure antigens and the sequence of nucleic acids. Loesh and coworkers (10) compared different microbiological tests by testing 204 plaque samples on 4 species of periodontal pathogens by means of culture tests, immunological tests, DNA probes and BANA test. The bacterial culture was least reliable, with accuracy of 61-79%, while the DNA probes were the most accurate methods, with reliability of 88-96% .

The use of nonselective and selective nutrient media contributes to the speed and better recognition and identification. With regard to their composition nonselective media differ according to their ability to promote the growth of certain groups of bacteria. Selective media, with the addition of an antibiotic, serve as isolation, i.e. gram negative rods. Thus TSBV is used in this way, a selective nutrient media, with the addition of an antibiotic and horse serum for isolation of *Actiobacillus actinomycetemcomitans* (11,12).

Enzyme tests

The knowledge that infection can be diagnosed by detection of enzyme activity directed towards proteins and peptides lead to the development of enzyme tests. Such an enzyme is BANA (N-benzoyl-DL-arginin-2naphthylamide). *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Treponema denticola* produce a trypsin-like enzyme, whose activity is measured by hydrolysis of the synthetic peptide, BANA. This reaction enables their detection. These tests indicate the presence of a group of periodontal pathogens by detecting their enzymes, such as colagenase, peptidase and trypsin-like enzymes, which destroy periodontal tissue. However, they do not enable detection of pathogens which do not have a defined enzyme profile, such as for example *Actinobacillus actinomycetemcomitans* (13,14).

Immunological tests

These tests are based on specific binding of monoclonal antibodies to the surface of the antigen of a particular microorganism. Fluorescent colours and radioactive isotopes are used to show the reaction antigen-antibody. Their main drawback is cross reactivity and inability to detect a pathogen for which there is still no antibody (13,14).

It would appear that there is one technique, immunoflurescent microscopy, which can reliably detect bacterial levels as low as 10x10 . Another effective technique, ELISA, leads to a colorimetric reaction of secondary binding antibodies and enzyme activity.

Molecular-diagnostic techniques DNA probes and PCR

DNA analytic method

The application of DNA oligonucleotide probes is based on a specific reaction of segments of one chain of nucleic acids with a complementary sequence of nucleic acid of the bacteria. The principle for detection of hybridisation products of a specific sequence of nucleic acid with a complementary sequence is most frequently radioactive or enzymic. The DNA probe is most often used for identification of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* i *Prevotellu intermediu* and other periodontal pathogens. The advantage of the method is that it enables high specificity and determination of an approximate number of tested pathogens, and detection as low as 103 bacteria in one sample. A disadvantage is the fact that some microorganisms have similar gens and determination of the sensitivity of the pathogen to the antibiotic is not possible (15). In 1994 Socransky and coworkers described the DNA-DNA hibridisation technique for identification of 40 subgingival species (16).

Polymerase chain reaction (PCR)

The chain reaction of DNA synthesis by means of DNA polymerase, or polymerase chain reaction (PCR), is an in vitro method of amplifying DNA, during which the gene sequences are selectively amplified. A precondition for carrying out the reaction is knowledge of the sequences of the nucleotide marginal areas of the DNA segments, on the basis of which the initial oligonucleotides are constructed (primer) and the existence of at least one initial DNA molecule which has the role of a template in the reaction. The basic PCR cycle consists of three steps which are performed in the same closed test-tube, at different temperatures. The PCR method is considered the fastest and most sensitive method available for detecting the presence of bacterial DNA sequences. Application of this method in the identification of periodontal microorganisms has been reported in recent papers (17,18).

Conclusion

Genetic, immunologic and enzymatic- based bacterial test are rapid and cost effective alternatives for evaluating periodontal infective organisms.

The main disadvantage of the specific methods is that they are available for only a small number of pathogens. Further more, they do not allow antibiotic susceptibility testing. Commercial diagnostic test for rutine use, similar to laboratory tests, will be widely used for assessing sites at risk and for monitoring the response to therapy.