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An Approach to the Microbiological Diagnosis of Chronic Periodontitis: An Overview.

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Review Article

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ABSTRACT

Periodontal diseases are diagnosed traditionally by a radiographic and clinical examination. An important disadvantage of diagnosis by this method is that it is difficult to differentiate the stable patients and patients with active disease. The clinical parameters are not sufficient to predict active periodontal disease. Improved knowledge in understanding the pathogenesis of periodontal diseases has lead to the development of various newer diagnostic tests. These can accurately measure the sub-gingival microbial challenge and the host responses to these challenges. The concept of diagnosing the disease with laboratory tests is relatively new to the dental sciences. These tests are useful to predict patients with active sites and to predict which tooth or site will experience clinical attachment loss in near future. In addition diagnostic tests help to categorize patients into different disease categories; i.e. aggressive or chronic periodontitis, they will also help in differentiation of patients who respond or do not respond to treatment. They can also be used to determine the prognoses of either the entire patient or a specific tooth. This review article will help the dental practitioners to rethink about the role of the laboratory in the assessment of chronic periodontitis.

INTRODUCTION

The diagnosis of chronic periodontitis is difficult as it is a multi-etiological disease. No single pathogen is responsible as the cause of the disease. Traditionally chronic periodontitis is diagnosed on the basis of radiography and clinical examinations. Clinical examinations include assessment of plaque, gingival inflammation, probing depth, and attachment loss. These methods make it difficult to differentiate stable patient from patients with active disease. There are various newer diagnostics tests available to diagnose the periodontal diseases which can accurately measure the sub-gingival microbial challenge and the host responses to these challenges. The concept of diagnosing disease with laboratory tests is under-utilized by dental professional in general.

With the help of laboratory tests it is easy to categorize patients into aggressive or chronic periodontitis. They also help in differentiation of patients who respond or do not response to treatment. They can also be used to determine the prognoses of either the entire patient or a specific tooth.

Microbiological Tests

Various diagnostic methods are used to identify the presence of specific periodontal pathogens includes anaerobic culture, immunofluorescence, Polymerase chain reactions (PCR) and DNA probe technology. The bacteriological culture methods have an additional advantage of making available the antibiotic sensitivity of the pathogen, which is useful in guiding treatment strategies.

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Specimen collection

Specimen collection is the most critical aspect of any microbiological diagnosis. Several techniques have been employed for specimen collection in the diagnosis of periodontal diseases. The choice of technique depends upon the objective of the treating dental surgeon. In the diagnosis of periodontal diseases the most common specimen which is collected is gingival crevicular fluid.

Collection method for GCF

Washing method

In this technique the gingival crevices is perfused with an isotonic solution (e.g. Hank's balanced salt solution) with a fix volume which helps in harvesting cells. The disadvantage is that it is complicated and technically difficult [1].

Capillary tubing or micropipettes

After isolation and drying of the site, a capillary tube of known diameter is inserted into the entrance of gingival crevice and GCF automatically transferred with capillary action into the tubes [2]. Advantage of this method is that it provides an undiluted sample whose volume can be assessed. The disadvantage is that the volume is not sufficient and it is difficult to remove complete sample from the tubes.

Absorbent filter papers

In intra-crevicular technique a filter paper strip is inserted into the gingival crevices whereas in extra-crevicular technique the strip is overlaid on the gingival crevice region to minimize the trauma. Intra-crevicular method the strip insertion is just at the entrance of the crevice or into the periodontal pocket till the base of the pocket is reached or until minimum resistance is felt. Advantage includes quick and easy to use, can be applied to individual site and less traumatic. The disadvantage of this method is that there is loss of GCF due to evaporation [3,4].

Several other methods such as pre-weighted twisted threads, intra-crevicular washings, micro syringes, plastic strips, platinum loops or micro-spatulas have been used for sample collection [5,6,7,8,9,10,11,12,13]

Diagnostic methods

Microscopic examination

Gram staining performed on direct smear prepared from specimens (e.g. GCF) helps in preliminary identification of periodontopathic bacteria based on their typical morphological characteristics.

Aerobic culture techniques

Aerobic culture techniques will help in the diagnosis of aerobic etiology which is also responsible for periodontal diseases [14]. It is easiest method for the cultivation of aerobic etiology with the help of common media like sheep blood agar and MacConkey agar and incubated aerobically at 37^0c for 24 to 48 hours. Based on the colony morphology, identification tests, the organisms can be easily identified and antibiotic sensitivity can be performed.

Anaerobic culture techniques

For cultivation of anaerobic bacteria evacuation – replacement jars, anaerobic jars with gas generators etc are used [15, 16].

Anaerobic environment is generated with the help of removal of oxygen by evacuation and replacing with hydrogen gas or also with the help of gas packs. The anaerobic bag culture set which includes a plate of anaerobic blood agar within the O_2 impermeable bag. The advantage of this system is that it contains its own gas generating kit and cold catalysts [17].

Incubation of cultures

After streaking, plates are kept in jar and evacuation – replacement is done and incubated for at least 48 hours and re-incubated for another 2-4 days to allow slow growing anaerobic bacteria. For the primary isolation 35 to 37^0c is the temperature which is essential for cultivation of anaerobic bacteria from clinical specimens. Different

culture media are used for cultivation of anaerobes like kanamycin blood agar, etc [18]. A suitable indicator system such as cultures of strictly aerobic organisms like Pseudomonas spp. or dye like methylene blue should be used to confirm the generation of an anaerobic environment.

Inspection of growth and identification

After incubation period is over the colonies are carefully inspected and tested for their aero tolerance. All strict anaerobes are identified by standard biochemical reactions. Gas liquid chromatography can also be used for detection of various metabolic products produced by the anaerobic bacteria [19].

Benzoyl-DL-arginine-naphthylamide (BANA) test

Periodontal diseases are most commonly associated with the bacteria namely *P.gingivalis, T. denticola and B. forsythus*. These bacteria can hydrolyze the synthetic trypsin substrate Benzoyl-DL-arginine-naphthylamide (BANA). This test is useful in detection of these bacteria from the samples taken from sub gingival sites in affected patients [20, 21].

Molecular techniques

Molecular techniques like polymerase chain reaction (PCR), Multiplex PCR, 16s rRNA detection are used to identify anaerobic bacteria and herpes viruses [22, 23].The most commonly used molecular technique for periodontal pathogens is polymerase chain reaction which is based on DNA probe technology. This technology has several advantages over culture techniques, including increased sensitivity and elimination of the need for viable bacteria. The sampling technique for these assays is relatively non-invasive, using either endodontic paper points or a sterile curette to obtain pathogens from the subgingival environment.

A number of bacteria have been studied for their relationship to “active” periodontal disease. Haffajee and Socransky showed the “red complex” has the strongest evidence of an association with periodontal disease progression. The“red complex” includesbacteria such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola*. In addition to these bacteria, *Aggregatibacter actinomycetemcomitans* has been found to be associated with aggressive periodontitis [24, 25].

Detection of specific interleukins

Tests to know the host response have been developed which improves understanding of the inflammatory and immune responses in periodontal disease progression[26]. Analysis of the host response for diagnostic purposes has involved the quantification of specific host-derived molecules within gingival crevicular fluid, serum, or saliva [27].

Gingival Crevicular Fluid Assays

Gingival crevicular fluid (GCF) is a serum transudate found in the gingival sulcus. In GCF, substantial numbers of polymorpho-nuclear leukocytes and epithelial cells as well as bacterial cells are found. Components of GCF are eluted off the strip and then analysed by either an immunologically based assay (enzyme-linked immunoabsorbence assay or ELISA) or by an enzyme-substrate assay [28]. These highly sensitive assays are able to quantify the presence of specific molecules in the GCF.

Various molecules are identified, but not all of them are associated with disease activity. Some of these molecules that have confirmed significant association with periodontal disease are discussed below.

Markers of Cell Death

Aspartate aminotransferase (AST) is an enzyme found in the cytoplasm of cells which is used as a degree of cell death [29]. Detection of these enzymes can be used as a diagnostic tool in phases of periodontal destruction from the GCF. Chambers et al [30] reported that elevated levels of AST were associated with a 9 to 16 time greater risk of experiencing active periodontal tissue destruction.

Markers of the acute inflammatory response

In polymorphonuclear leucocytes (PMN) lysosomal granules,ß-glucuronidase and elastase are found. Levels of these enzymes are linked to the degree of acute inflammation present in the periodontium and gingival sulcus. Subjects with elevated levels of GCF ß-glucuronidase were 6 to 14 times more likely to experience significant clinical attachment loss in the next 3 months than patients without elevated levels of ß-glucuronidase.

Elastase was found to be elevated in patients with active periodontal disease [31, 32, 33]. Matrix metalloproteinases (MMPs) released by inflammatory cells have also been shown to aid in the diagnosis of periodontal diseases [34].

Pro-inflammatory Cytokines

Cytokines released controls the functions of a wide variety of cells and are involved in regulating the immune and inflammatory response. Large number of cytokine has been discovered but the most promising cytokine which can be used as diagnostic marker is interleukin-1ß(IL-1ß) [35, 36]. These cytokines exerts various biologic effects, including initiating the acute phase response to infection. However, in the periodontium, it mediates destruction of connective tissue and bone resorption [37]. GCF levels of IL-1ß have been found to be consistently associated with the severity of periodontal disease [38].

Immunoglobulin

Immunoglobulins are produced in periodontal disease [39]. Immunoglobulins G (IgG) arenot associated consistently with periodontal disease, although subtypes of IgG may be found in subjects at higher risk for periodontal disease progression [40]. Remarkably, immunoglobulin A (IgA) has been shown to be elevated in healthy subjects and subjects who have a decreased rate of periodontal disease progression, suggesting that it may be a marker of protection [41, 42]. Serum can be used to measure the levels of antibodies to specific periodontal pathogens. Several investigators [43, 44] have found that serum antibodies to periodontal pathogens are generally elevated in patients with periodontal disease but few patients fail to respond with an appropriate antibody response.

Salivary enzymes

The ability of ß-glucuronidase in saliva is used to differentiate patients by levels of periodontal probing depths and gingival inflammation. Although preliminary studies have been promising, no long-term studies evaluating the use of a marker for periodontal disease progression in saliva have been performed [45].

At present the endpoint of periodontal therapy is based on the reduction of probing depth and gingival inflammation. However, more sensitive measures of periodontal infection exist, including analysis of post-treatment levels of periodontal pathogens as well as a reduction in the host response to these pathogens e.g. patients who continue with high levels of periodontal pathogens or high levels of inflammatory mediators in their saliva can be treated additionally with antibiotics. The use of periodontal diagnostic tests to determine the conclusion of the “active” phase may help reduce the rate of refractory disease.

The present overview is being written to increase awareness amongst dentists as to the availability of diagnostic tests for monitoring periodontal disease and they must have a clear considerate of the relationship between periodontal diagnostic testing and treatment as well as an appreciation of the financial and health benefits of diagnostic testing. In addition to being used to diagnose a disease, diagnostic testing can determine when a chronic, persistent disease has been adequately treated.

To conclude the laboratory tests with clinical diagnosis will help in understanding the chronic periodontitis which is a multi-factorial infectious disease.

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