REVIEW ARTICLE

Chair side diagnostic kits in Periodontics

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Abstract

A good clinical diagnosis is the need for the hour. Proper diagnosis is essential for better treatment planning of the disease. Traditional clinical measurements used for periodontal diagnosis are often of limited usefulness as they are indicators of previous periodontal disease rather than present disease activity. Hence, there is a need for developing novel diagnostic kits that can detect active disease, predict future disease emergency or progression and evaluate response to periodontal therapy, therapy facilitating management of periodontal patient. In this modern era there has been tremendous research in the field of diagnostic tools that can be used by periodontists in their daily practice. Different chair side diagnostic kits are discussed in this review which would be helpful for proper diagnosis, evaluating a disease prognosis and proper treatment planning.

Keywords: Periodontal disease, diagnostic, chair side tests.

Introduction

"A correct diagnosis is three fourths the remedy" – M. K. Gandhi. Diagnosis is the identification of a condition, disease, disorder, or problem by systematic analysis of the background or history, examination of the signs or symptoms, evaluation of the research or test results, and investigation of the assumed or probable causes. Effective prognosis is not possible without effective diagnosis.

Periodontitis is a set of inflammatory diseases affecting the periodontium, i.e., the tissues that surround and support the teeth. Periodontitis involves progressive loss of the alveolar bone around the teeth, and if left untreated, can lead to the loosening and subsequent loss of teeth. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an over-aggressive immune response against these microorganisms. A diagnosis of periodontitis is established by inspecting the soft gum tissues around the teeth with a probe (i.e., a clinical examination) and by evaluating the patient's X-ray films (i.e. a radiographic examination), to determine the amount of bone loss around the teeth.[1]

"Periodontal Diagnosis" is an important tag that a clinician ties on the periodontal disease condition of the patient, capturing all his past experience with the condition in question. The entire constellation of signs and symptoms, along with a detailed history is elicited, documented and interpreted to reach at a diagnosis. Most often an accurate diagnosis is, the very first concrete step towards the planning and execution of an appropriate individualized treatment plan, contributing significantly towards the success of the therapy [2.]

It is relatively easy to detect significant anatomical damage, caused by advanced stage of periodontal disease with conventional diagnostic aids. On the other hand early or incipient disease detection serves as a challenge even for the most experienced clinician. Emphasis on early detection serves as an impulse for managing the disease with minimally invasive and economic therapeutic modalities. Hence, there is a need for developing novel diagnostic kits that can detect active disease, predict future disease emergency or progression and evaluate response to periodontal therapy, therapy facilitating management of periodontal patient. The available chair side diagnostic kits are designed to measure microbiological, immunologic and genetic constituent in oral diagnostic fluids

namely; saliva and gingival crevicular fluid (GCF), indicative of health or disease.

These tests are technique sensitive making harvesting of a sample and reproducing the results a tedious task. Also, the specificity and sensitivity of these diagnostics is fundamentally limited by their inability to simultaneously measure the local concentration of multiple biomarkers. Hence rapid chair side diagnostic tests should be developed that provide maximum information with minimal technicalities.

More than 200 species of microorganisms colonize the oral cavity, but only a few of these are thought to be pathogens. [3] Among the subgingival bacterial species identified so far, Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi) and Aggregatibacter actinomycetemcomitans (Aa) have been associated with progressive periodontitis[4]. Aa is infrequently found in periodontally healthy individuals [5], whereas Pi has been found in either healthy subjects or patients with gingivitis [6]. Elevated levels of these putative pathogens may be useful indicators of both active periodontitis and increased risk of gingival attachment loss.

The goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location and severity. Traditional clinical measurements (probing pocket depth, bleeding on probing, clinical attachment loss, plaque index, radiographs) used for periodontal diagnosis are often of limited usefulness in that they are indicators of previous periodontal disease rather than present disease activity.

The ideal diagnostic test should be [7, 8]:

1. Highly specific, sensitive, reproducible and quantitative.
2. Simple to perform, rapid, one-stage or a two-stage procedure.
3. Non-invasive.
4. Versatile in terms of sample handling, storage and transport.
5. Amenable to chairside use.
6. Economical.

Objectives of chair side tests

1. They are minimally invasive, thus having an edge over conventional diagnostic aids.
2. Relatively less tedious to the patient as the appointment time is reduced.
3. Less cumbersome or technique sensitive, making them user friendly.
4. Help in early diagnosis and treatment planning.
5. Can be used as an encouragement tool to motivate the patient.

Several methods have been employed to detect putative periodontopathogens in clinical samples. These include cultural methods, microscopy, immunofluorescent assays, enzyme-linked immunosorbent assays, trypsin-like protease assays, DNA probes [9] and the PCR [10].

Among these tests, chairside periodontal kits provide immediate reports of the microflora associated with the disease compared to cumbersome and time-consuming traditional laboratory procedures. Chairside periodontal test kits can be categorized as

1. Microbiological test kits
2. Biochemical test kits
3. Genetic kits.

1. Microbiological test kits

Enumeration and identification of the microflora of the periodontal pocket have been an important part of research efforts for many decades. It is thought that as many as 300 distinct bacterial species can inhibit the periodontal pocket. Many of these have not been identified.

The microbiological tests have the potential to support the diagnosis of various forms of periodontal disease, to serve as indicators of disease initiation and progression and to determine which periodontal sites are at higher risk for active destruction. The bacteriological tests (Microscopy, Culture, Omnigene, Affirm DP and Evalusite) are mainly aimed at spirochetes, Aa, Pg and Pi. Microbial tests can also be used to monitor periodontal therapy directed towards the suppression or eradication of periodontopathogenic organisms.

Omnigene

These are DNA probe systems for a number of known periodontopathogen subgingival bacteria. OmniGene Diagnostics, Inc. has applied the principles of genetic engineering to develop species-specific DNA probe tests for eight periodontal pathogens (Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetem-comitans, Fusobacterium nucleatum, Eikenella corrodens, Campylobacter rectus, Bacteroides forsythus, and Treponema denticola). The test requires minimal effort on the part of the clinician: subgingival plaque samples are collected from the patient and sent through the mail for analysis by OmniGene Diagnostics' fully licensed clinical reference laboratory. Results are transmitted to the practitioner by phone, fax, or mail. The use of diagnostic tests for periodontal pathogens is a relatively new concept in dentistry and acceptance of the OmniGene Diagnostics tests by the dental marketplace has been slower than anticipated. OmniGene Diagnostics' challenge for the future is to persuade the dental community that monitoring periodontal pathogen levels, as well as other clinical indicators of disease, is essential to providing optimal care to the periodontitis patient.[11]

Merits

1. Reports are provided within short periods of time, few hours to few days.
2. It helps in identification of number of known periodontal pathogen[12]

Figure 1- Omnigene

Evalusite (Kodak)

It is a novel membrane immunoassay commercially available in Europe and Canada for the Chairside detection of 3 periodontal pathogens. It involves linkage between the antigen and a membrane bound antibody to form an immunocomplex that is revealed through a calorimetric reaction. The patient plaque sample is prepared by the addition of a detergent, mixed and then squeezed through a filter into a reagent well. Membrane bound antibody in the well specific to A.actinomycetemcomitans, P.gingivalis and P.intermedia reacts with plaque sample. Antigen and antibody complexes formed on the membrane are detected by the addition of an enzyme-labelled second antibody together with a colored enzyme substrate. Separate dots indicate the presence of 3 different species.[13]

Merits

It employes a normal membrane base enzyme immunoesaay for the detection of three putative periodonto pathogens. (Aa, Pg, Pi).

Demerits

1. It is multistage test.
2. It has a subjective calorimetric end point.
3. There is no permanent record of the result.
4. Gives the assumption that the three organisms are causing the disease. [14]

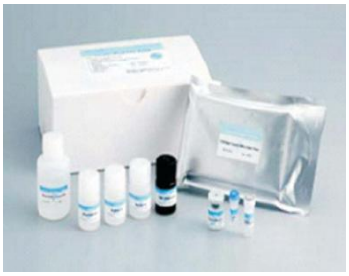

Figure 2 - Evalusite

PerioScan®

Perioscan is a diagnostic test kit that utilizes the BANA (N-benzoyl-DLarginine- 2 naphthylamide)-hydrolysis reaction, developed to detect bacterial trypsin-like proteases in the dental plaque. The microbial-enzymatic BANA test is one of the modern alternatives to bacterial cultures. It detects the presence of three periodontal pathogens in the subgingival plaque (T. denticola, P. gingivalis, and B. forsythus).

The BANA test (Figure 3) was developed by Dr. Walter Löesche and co-workers at Michigan University, being the result of more than 15 years of research. Of the 60 bacterial species studied in the subgingival microbiota, only the anaerobic bacteria Porphyromonas gingivalis, Bacteroides forsythus and Treponema denticola possess a trypsin-like enzyme, which hydrolyzes the synthetic peptide benzoyl- DL-arginine-naphthylamide or BANA. The test can detect the presence of these three anaerobic species, without being able to differentiate them[15].

The BANA test is very sensitive, detecting small quantities of pathogens. No meaningful differences could be found between DNA probes, immunological reagents and the BANA test, when seeking to detect these species in plaque samples removed from periodontal disease patients.[16,17,18]

The test can be used for assessment of oral halitosis, to detect the presence of two BANA positive species on the tongue surface: Stomatococcus mucinlagenous and Rothia dentocariosa.

Figure 3 - BANA Test

Principle of BANA test

Peptidases of these three bacterial species (T. denticola, P. gingivalis, and B. forsythus) can hydrolyze the peptide analog N-benzoyl-DL-arginine-2 naphthylamide (BANA). One of the hydrolytic products of this reaction is B-naphthylamide, which reacts with a reagent, which is imbedded in the upper strip of the test, producing a permanent blue color. Blood and saliva do not interfere with the test [19].

The BANA test is a plastic strip to which two separate reagent matrices are attached. The lower white reagent matrix is impregnated with N-benzoyl- DL-arginine-B-napthylamide (BANA). Subgingival plaque samples are applied to this lower matrix. The upper buff reagent matrix contains a chromogenic diazo reagent, which reacts with one of the hydrolytic products of the enzyme reaction, forming a blue color. The blue color appears in the upper buff matrix and is permanent. The intensity of the color determines whether it is a positive or weak reaction.

Directions for use

Anaerobic microorganisms associated with periodontal disease are found in the subgingival plaque. To obtain specimens for testing, sites should be cleared of supragingival plaque. A Gracey curette may be used to obtain subgingival plaque specimens, which are placed on the lower matrix. Four teeth should be sampled in each subject. Before taking another specimen, wipe the curette on a clean piece of cotton or other suitable wipe to prevent carry-over of plaque. Then the upper matrix is moistened with saline solution and the test is folded so as the two matrices are coming in contact. It is incubated for 5 minutes at 55 Celsius degrees temperature. If BANA positive species are present when the test is opened, a permanent blue coloration on the upper matrix is found. The higher the concentration of bacterial species, the darker blue coloration is present on the test. According to the result, the test can be positive, weak positive, or negative.

Merits

Used to identify volatile sulphur compounds in halitosis patients

Demerits

1. In this test, there is always a lack of quantitative data.
2. The specific bacteria that are responsible for enzyme production can't be determined.
3. They cannot identify the presence of other pathogens that do not produce trypsin like enzyme.
4. The results are qualitative and rely upon the operator's assessment at the calorimetric end point.[20,21,22]

2. Biochemical test kits

Biochemical test kits used in periodontics analyze the gingival crevicular fluid (GCF). Since this fluid is derived from periodontal tissues, evaluating its constituents such as host-derived enzymes, inflammation mediators and extracellular matrix components may provide early signs of alterations.

Perio 2000

The Diamond Probe/Perio 2000 System is a periodontal probe that combines advanced ion selective electrode technology with the standard "Michigan O" style probe

The Diamond Probe/Perio 2000 System is a periodontal probe that combines advanced ion selective electrode technology with the standard "Michigan O" style probe. It is intended to measure probing depths, to evaluate the presence or absence of bleeding or probing, as well as to detect the presence of sulfides in periodontal pockets. The sulfide sensor component in the system is used as an adjunct to traditional diagnostic techniques in the evaluation of periodontal diseases in adult patients.

The system consists of:

1. Single use, disposable sensor tips that combine an updated standard Michigan "O" style dental probe with a sulfide sensor for use during one-time examinations
2. An electronic control unit that provides real-time visual feedback of bacterial activity to the practitioner and patient
3. Probe handle, hand-piece cable, foot switch, and external power supply
4. Wash solution
5. Accessory stand for convenient wash cup placement and temporary probe storage
6. System check

It can be used:

1. During initial patient screening as an adjunctive measurement of a patient's oral health status
2. During and after routine supportive periodontal therapy
3. At maintenance intervals
4. As a tool to provide patient education and motivation.[23]

Figure 4 - Perio 2000

Prognos-Stik

This test kit was released in the year 1993. It detects elevated levels of MMPs in the gingival crevicular fluid such as the elastases. The GCF is collected onto the filter paper strip impregnated with a known amount of buffered elastase substrate labeled with a fluorescent indicator. Elastase on the test strip cleaves the substrate during the reaction time of 4-6 minutes and releases the indicator, visible under fluorescent light. Elastase is released from the lysosomes of polymorphonuclear leucocytes which accumulate at sites of gingival inflammation. The presence of elevated levels of

elastase in GCF may thus be indicative of active disease sites [24.] Although a relationship between elastase levels in GCF and periodontal disease activity has been reported, the position is still far from clear. Further clinical trials are needed before the value of this test kit in clinical practice can be ascertained.

Periocheck

Periocheck (Advanced Clinical Technologies Inc., Westwood, MA 02090, USA) is a rapid chairside test to detect the presence of neutral proteases. Periocheck has FDA (Food and Drug Administration) approval in the United States. The presence of these enzymes has been implicated in collagen breakdown which is an important feature of periodontal disease. Crevicular fluid is collected on filter paper strips and these are placed on a collagen dye labelled gel matrix. Soluble dye-labelled fragments of collagen are formed from the reaction of neutral proteases with the gel and these diffuse onto the sample strip turning the papers colour to blue. The quantity and intensity of the colour reaction is compared to a standard colour chart and is related to the level of neutral protease activity originally present in the crevicular fluid sample.[25] The test is only qualitative and not specific for PMNL collagenase, which is thought to be the dominant collagenase at active sites [26]. Indeed, a high proportion of the enzyme is likely to be bacterial in origin. Furthermore, interproximal sites cannot be sampled, due to the risk of saliva contamination, and this is clearly a major drawback with this method. It is the most rapid chairside test for neutral proteases in GCF like elastases, proteinases and collagenases. The levels of these enzymes in GCF have been noted to increase with the development of gingivitis as well as sites of established periodontitis.

PerioGard

PerioGard is based on the detection of an enzyme called aspartate aminotransferase (AST). AST is a soluble intracellular cytoplasmic enzyme that is released from within the cell upon its death. Since cell death is an important part of periodontal pathogenesis, AST levels in GCF have great potential as markers of early periodontal tissue destruction. Elevated total AST levels in a 30-second sample have been positively associated with disease-active sites in contrast to inactive sites [27,28]. This commercial test consists of a tray with two test wells for each tooth, and appropriate reagent for conducting the test. The test involves collection of GCF with the filter paper strip which is then placed in tromethamine hydrochloride buffer. A substrate reaction mixture containing 1-aspartic and α-keto-gluteric acid is added to the sample and allowed to react for ten minutes. In the presence of AST, the Aspartate and α keto-gluteric acid are catalyzed to oxaloacetate and glutamate. The addition of a dye such as fast red results in a color product, the intensity of which is proportional to the AST activity in the GCF sample. In practice, the PerioGard assay suffers from poor differentiation between colors and is a relatively complex procedure involving multiple steps.

PerioWatch

The PerioWatch was developed as a simple method of analyzing Asparate amino Transferase (AST) at the chairside. The principle of this test is that, in the presence of pyridoxal phosphate, AST catalyzes the transfer of an amino group from cysteinesulfinic acid, by a aketoglutaric acid to yield β-sulfinyl pyruvate and glutamate. β-sulfinyl pyruvate rapidly decomposes and releases inorganic sulfite which react with malachite green to convert from a green dye to colourless form. The rate of conversion of malachite green is directly proportional to the AST concentration.

3. Genetic test kits

Various gene polymorphisms are considered to be risk factors for the initiation or progression of periodontal disease. In 1997, Kornman et al. [29] found an association between the polymorphism in the genes encoding for interleukin-1α and interleukin-1β and increased severity of periodontitis. Identification of the genetic polymorphism is difficult but now some chairside kits are available for its detection.

PST Genetic Susceptibility Test

Ever wonder why some people with loads of plaque don't get attachment loss and others with good hygiene are losing teeth? Common sense and more recently science indicate that there is an inherited component of periodontal disease that is very important in determining disease behaviour. Clinicians have recognized for years that some individuals get lots of plaque and calculus, but experience little loss of periodontal support. Other patients seem to have really clean mouths, but experience extensive bone loss. Their disease is not so much related to the presence of bacteria but to some other factor. Within the past two years, a genetic marker has been identified that can be correlated with the degree of tissue destruction that occurs in some periodontal patients with advanced disease. Conventional forms of therapy such as cleaning the gingival pockets etc. often, experience has shown, fail to be effective. An American research team was able to show for the first time that these patients to a disproportion extent (> 50 %) had a genetic defect in a certain component of the immune system. This leads to over-production of an important local inflammatory mediator in the immune system, namely interleukin-1 (IL-1). The

over production of IL-1 leads to a strong immuno-response in the bone and connective tissue even when only small amounts of bacteria are present. As a result, a hyper-activation of so called osteoclasts can be detected which themselves then cause an aggressive bone resorption.

Genetic Principals of the GenoType® PST®

Two polymorphisms within the IL-1 gene cluster show a close association with periodontitis:

1. Interleukin 1A gene, position -889
2. Interleukin 1B gene, position +3953

Within both polymorphisms allele 1 harbors a cytidin (C), whereas allele 2 carries a thymidin (T) at the respective position. In particular, when both genes carry allele 2 a strong over-production of the local inflammatory mediator, interleukin-1 will occur.

The GenoType® PST® detects the corresponding allele combination in patients allowing an evaluation of the individual periodontitis risk and future strategies for therapy.

PST (Genetic Susceptibility Test) is the first and only genetic test which analyzes two IL-1 genes for variations that identify an individual's predisposition for over expression of inflammation and risk for periodontal disease.IL-1genetic susceptibility may not initiate or cause the disease but rather may lead to earlier or more severe disease.

Indications for the GenoType® PST® test:

1. Patients exhibiting aggressive, therapy-resistant periodontitis for individual therapy planning
2. Patients with established periodontitis and loss of attachment for progress assessment
3. Relatives of PST®-positive patients for risk assessment before major restorative therapy and optimization of prophylaxis and recall interval.

Conclusion

In Periodontology, the success of any treatment is dependent upon the accuracy of the initial diagnosis. At present, the majority of chronic periodontitis cases can be adequately managed using existing diagnostic methodology, although it is clearly more desirable to be able to diagnose “active disease” as soon as it occurs, rather than months later. There is an ongoing transition in periodontal disease diagnosis from clinical examination to more accurate advanced diagnostic methods. The availability of chairside diagnostic Test Kits will aid in early diagnosis and treatment. It also helps in improving patient's compliance towards periodontal treatment considerations. The newer commercially available chairside tests for host and bacterial markers of periodontal disease offer exciting prospects which would make the monitoring of specific sites an attainable goal in near future.

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