

CHAIRSIDE DIAGNOSTIC TEST KITS IN PERIODONTICS - A REVIEW

Sachin Malagi*

Abstract

Traditionally, periodontal diseases have been diagnosed by radiographic and clinical exams, including the assessment of plaque with a plaque index (PI), gingival inflammation with the bleeding on probing index (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL). Nowadays, clinical chairside tests are in use for more precise molecular diagnostics and treatments. This review describes some diagnostic test kits on the market that can facilitate the clinical exams and the establishment of a diagnosis.

Keywords: Periodontal disease – gingival inflammation – diagnostic – chairside tests.

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KITS DE TEST DE DIAGNOSTIC EN PARODONTOLOGIE – UNE REVUE DE LA LITTÉRATURE

Résumé

Traditionnellement, les maladies parodontales sont diagnostiquées par des examens radiologiques et cliniques en utilisant certains indices pour bien déterminer la sévérité et l'importance des atteintes : indice de plaque (IP), indice de saignement (IS), profondeur de poche (PP) et niveau d'attachement (NA). Un inconvénient majeur de ces évaluations est leur incapacité de déterminer les phases actives ou stables de la maladie parodontale. Actuellement, des tests cliniques peuvent être utilisés au cabinet dentaire pour des diagnostics moléculaires plus précis permettant ainsi des diagnostics précoces et des traitements plus prompts. Ce papier est une revue descriptive de quelques tests disponibles sur le marché dont l'usage facilite l'examen clinique et l'élaboration d'un diagnostic.

Mots-clés : maladie parodontale – inflammation gingivale – diagnostic.

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* MDS

Lecturer, Dpt of Periodontics

COORG Institute of Dental Sciences

Rajiv Gandhi University of Health Sciences, India

perio2_007@yahoo.com

Introduction

Periodontitis is a prevalent disease of men characterized by loss of connective tissue attachment and bone around the teeth, in conjunction with the formation of periodontal pockets due to apical migration of the junctional epithelium. Periodontal disease progression is episodic [1] and site-specific [2].

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]. Consequently, elevated levels of these putative pathogens may be useful indicators of both active periodontitis and

increased risk of gingival attachment loss. However, knowledge of how their numbers relate to disease progression is still unclear and for longitudinal studies accurate assessment of their numbers in clinical samples is needed. The goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location and severity. These findings serve as a basis for treatment planning and provide essential data during periodontal maintenance and disease

monitoring phases. 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 1990s have seen the emergence of a multitude of diagnostic tests based on physical, chemical, microbiological and immunological methodologies. The philosophy behind the emergence of such tests is that the earlier active disease is diagnosed, the less invasive, time consuming, and therefore costly the required treatment, and the better the long term prognosis for patients with destructive disease [7]. Furthermore, with the recognition that risk groups and unpredictable disease patterns exist, the benefits of objective testing for initial diagnosis and for the long-term maintenance of periodontal patients become clear. For periodontal diagnosis, the ideal diagnostic test should be [8]:

1. Quantitative.
2. Highly sensitive method capable of analyzing a single periodontal site in health as well as disease.
3. Reproducible.
4. Highly specific.
5. Simple to perform.
6. A rapid, one or two stage procedure.
7. Non-invasive.
8. Versatile in terms of sample handling, storage and transport.
9. Amendable to chairside use.
10. Economical.
11. Dependent upon simple and robust instrumentation. 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



Fig. 1: Omnigene.



Fig. 2: PerioScan®.

time-consuming traditional laboratory procedures.

Chairside periodontal test kits can be categorized as

- Microbiological test kits
- Biochemical test kits
- Genetic kits

Microbiological test kits

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 (Fig. 1)

These are DNA probe systems for a number of known periodontopathogen subgingival bacteria. A paper point sample of sub-gingival plaque is placed in the container provided and mailed off to the company for assay. Probes are available for the detection of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *F. nucleatum*, *C. rectus*, *T. denticola* and *E. corrodens*. Reports are provided within very short time periods (few hours to few days).

EvaluSite

EvaluSite is a kit that employs a novel membrane-based enzyme immunoassay for the detection of three putative periodontopathogens: Aa, Pg and Pi. A sub-gingival sample is collected using paper points and added to a sample tube. The eluent is then added to the kit, which employs a sandwich-type ELISA (enzyme-linked immunosorbent

assay); a pink spot is displayed if the test organism is present. The main weaknesses of this test kit reside in 1) the assumption that the three detected organisms are causing disease; (2) it is a multistage test; (3) it has a subjective calorimetric end point and (4) there is no permanent record of the results [11].

PerioScan®

Perioscan is a diagnostic test kit that utilizes the BANA (N-benzoyl-DL-arginine-2-naphthylamide)-hydrolysis reaction, developed to detect bacterial trypsin-like proteases in the dental plaque (Fig. 2). A trypsin-like activity has been identified in strains of *P. gingivalis*, *T. denticola*, *T. forsythia* and some *Capnocytophaga* strains [12, 13]. BANA is an example of a substrate-conjugated beta-naphthylamine (p-NA), which is hydrolyzed by this trypsin-like enzyme to release free p-NA. The latter is a chromophore and reacts with a variety of dyes (e.g. Fast-Garnet GBC) to produce colored products.

Subgingival plaque is collected and placed on a BANA-containing strip, which is then folded to contact a second strip containing the "Fast-Black" dye reagent. The folded card is placed inside an oven for 15 min at 55°C and any blue-black color that appears is scored positive for the above species. The sensitivity of the method has recently been improved [14]. The main disadvantage of this technique is that it relies upon plaque sampling and assumes that the test organisms identified as being present signify active disease. This is known not to be the case for all patients and sites. Furthermore, results are qualitative and rely upon the operator's assessment of the calorimetric end point.

One of the potential difficulties of this test is that it may be positive at clinically healthy sites and might remain so after treatment.

The problem with all microbiological tests is that it is likely that a range of interacting bacteria is capable of causing disease, the combination may

differ between individuals, and tests directed at individual species may be too specific, and may not account for different clonal types.

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

Various pathogenic microorganisms like *P. gingivalis*, *P. intermedia* and *T. forsythia* produce sulphates, thereby significant levels of volatile sulphide compounds (VSCs) by degradation of serum proteins: cysteine and methionine. Since these VSCs can directly degrade periodontal structures aggravating periodontitis, their evaluation can indicate the subgingival microbial load. Perio 2000 system is designed to display the sulphide level digitally at each site. In brief, the probe tip should be hydrated using sterile wash solution provided by the manufacturer then inserted subgingivally at peak or hold operational mode. After a positive reading, the tip is washed and reinserted in other subgingival site.

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 [15]. 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.

Perio-Check

Periocheck has FDA (Food and Drug Administration) approval in the United States. It is reported to measure neutral protease activity within GCF. The GCF sample strip is placed on a gel containing insoluble dye-labelled collagen fibrils (remazobright blue-collagen substrate powder) and incubated. In the presence of neutral proteases (which diffuse from the strip into the gel), the insoluble collagen-dye complex is digested to release soluble dye-labelled fragments, which diffuse back into the strip, turning it blue. Again the test is only qualitative and not specific for PMNL collagenase, which is thought to be the dominant collagenase at active sites [16]. 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 chair-side 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 [17, 18]. 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 L-aspartic acid and α -keto-glutaric acid is added to the sample and allowed to react for ten minutes. In the presence of AST, the Aspartate and α -keto-glutaric 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 [19].

In practice, the PerioGard assay suffers from poor differentiation between colors and is a relatively complex procedure involving multiple steps.

Pocket Watch

The Pocket Watch was developed as a simple method of analyzing AST at the chairside [20]. The principle of this test is that, in the presence of pyridoxal phosphate, AST catalyzes the transfer of an amino group of cysteine sulfuric acid by α - keto- glutaric acid to yield β -sulfinyl pyruvate. Glutamate β -sulfinyl pyruvate spontaneously and rapidly decomposes and releases inorganic sulfite. The sulfite ion instantaneously reacts with malachite green (MG), simultaneously causing MG to convert from a green dye to its colorless form, thereby allowing the pink-colored rhodamine B dye to show through. The rate of conversion of MG is directly proportional to AST concentration. However, components of the extracellular matrix and its dissolved products are present in GCF of destructive pockets, and they may release sulfide ions. In brief, AST activity determined by Pocket Watch provides not only an index of cell death but of the extent of the destructive pockets.

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. [21] found an association between the polymorphism in the genes encoding for interleukin-1 α and interleukin-1 β 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

Periodontal susceptibility test (PST®) is the first and only genetic test that analyzes two interleukins (IL-1 α and IL-1 β) genes for variations. IL-1 genetic susceptibility may not initiate or cause the disease but rather may lead to earlier or more severe disease. The IL-1 genetic test can be used to differentiate certain IL-1 genotypes associated with varying inflammatory responses to identify individuals at risk for severe periodontal disease even before the age of 60. Clinically, PST® is used in [22]:

- New periodontal patients to assist in developing treatment plans.
- Patients requiring extensive periodontal and/or implant therapy to determine prognosis, improve patient acceptance and optimize treatment outcomes.
- Smoking patients as an additional incentive for smoking cessation.
- Maintenance patients to set recall intervals and improve compliance.
- Patients with early signs of disease to help determine the need for referral to a specialist.

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 it occurs, rather than months later.

Many of the biochemical chairside diagnostic test kits have been marke-

ted. The newly commercially available chairside tests for host and bacterial markers of periodontal disease offer prospects which would make the monitoring of specific sites possible. However, the clinician must ensure that the use of such tests will benefit the patients in terms of both the value of diagnostic data obtained and the cost in time and money.

Validation of novel periodontal diagnostics need to be benchmarked with existing gold standards of the disease, such as alveolar bone levels and clinical attachment levels, in large populations.

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