Recent advancement in clinical diagnosis

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Abstract

Periodontitis is a chronic inflammatory condition of the tissues surrounding the tooth, associated with attachment loss. It is a series of events which starts from gingivitis and when remains untreated progresses to periodontitis. Traditional periodontal clinical diagnostic parameters which include probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs are insufficient for identifying sites of active diseases. Thus, advanced techniques like newer generation of periodontal probes, advances in microbiological analysis, immunodiagnostic techniques, molecular biology techniques, biomarkers and genetic testing will improve the identification of gingivitis prior to the exhibition of symptoms of periodontitis.

Keywords: Probes; Immunodiagnostic; Biomarkers; Molecular biology technique; Genetic testing

1. Introduction

Periodontitis is a chronic inflammatory condition of the tissues surrounding the tooth which is generally associated with attachment loss. It is a series of events which starts from gingivitis and progresses to periodontitis.

Gingivitis is the inflammation of gingiva while periodontitis is an irreversible condition associated with both hard and soft tissue loss.1 Traditional periodontal clinical diagnostic parameters like probing depth, bleeding on probing, clinical attachment level, plaque index, and radiographs are easy to use, cost-effective, and are relatively non invasive but are limited to disease history and not current disease status or activity. These procedures are insufficient for identifying sites of active disease, for diagnosis and treatment planning, for monitoring patient’s response to therapy and for measuring the degree of susceptibility to future disease progression. New technology and advances in microbiology, immunodiagnostic techniques, molecular biology techniques, genetic testing and chair side kits help to diagnose more accurately and develop treatment plans for individual situation. Thus, adding more advanced techniques will improve the identification of gingivitis prior to the exhibition of symptoms of periodontitis, monitor progression and also avoid mismanagement of periodontal involved dentition.

2. Newer generation of probing system

2.1. First generation probes (conventional or manual periodontal probe)

University of Michigan “O” probe (figure – 1), University of North Carolina (figure – 2), Goldman Fox probe (figure – 3) and William’s probe. They are gold standard method, simple to use and tactile sensation of the periodontal structures can be felt but they have accuracy and reproducibility concerns, can over or underestimate pocket depths, and may be painful. Also they are not able to obtain 3-D information and a dental assistant is needed to record the data.
Figure 1 University of Michigan O probe

Figure 2 University of North Carolina-15 probe

Figure 3 Goldman Fox probe
2.2. Second generation probes (constant force probes): (figure – 4)
They are introduced by Hunter in 1994. These probes have a disposable hemispheric tip of 0.5mm diameter with a visual guide used sliding scale and two indicator lines that meet at a specified pressure. These probes are consistent, reproducible and reduces operator error.

But they may create errors in data readout, and recording. A dental assistant is always needed to record the data on the chart. They lack tactile sensation. They can over or underestimate pocket depth. Also they are unable to obtain 3-D information.

![Second generation probe](image)

**Figure 4 Second generation probe**

2.3. Third generation probes (constant force automated probes)
(Figure – 5) Foster Miller probe, Florida probe, Goodson and Kondon fiber optic probe and the Toronto automated probe are types of automated periodontal probes. They are consistent, reproducible and automated. These probes eliminate data read out and records error but they lack tactile sensation. Sometimes they may over or underestimate pocket depth. They are also unable to obtain 3-D information.

![Third generation probe](image)

**Figure 5 Third generation probe**

2.4. Fourth generation probes (ultrasonographic periodontal probe)
(Figure – 6) These ultrasonic probes involves projection of an ultrasonic beam with high frequency to the periodontal pockets. The echoes of the ultrasound wave reflected by the crest of the periodontal ligament are recorded by a transducer which is located inside the probe hand piece. These waves are transmitted to computer software for analysis. The ultrasonic image is constructed and the computer software translates the data to estimate periodontal pocket depth measurements. These probes are non-invasive and automatically records and reads out of the data but the echo waveform interpretation of pocket depth is difficult sometimes. Moreover they are costly, have poor contrast and feasibility for 3-D remains unclear.

![Fourth generation probe](image)

**Figure 6 Fourth generation probe**
2.5. Fifth generation probes
These probes are designed to provide a 3D image and are non-invasive. When probed the periodontal ligament, a narrow beam of ultrasonic energy is projected down between the tooth and the bone from a transducer, which is scanned manually along the pocket depth.

3. Non periodontal probes

3.1. Calculus Detection probe
It detects subgingival calculus by means of audio readings. This device has a lightweight, autoclavable handpiece, and it produces an audible beep to signify calculus detection. The probe also has a short waterline hookup, however, it is expensive and the handpiece is bulkier than a standard periodontal probe.

3.2. Diamond probe
It is plastic instrument with black bands for measurement of pockets and it also measures the volatile sulphur content within the sulcus, thus acting as an indicator of gram negative bacterial activity. This probe might detect periodontal disease at an early stage and might find an active site that requires treatment but the probing pressure is not controlled. Also periodontal disease caused by bacteria that do not produce volatile sulphur compounds are missed.

3.3. The Periotemp probe
It is a temperature sensitive probe, which detects early inflammatory changes in the gingival tissues by measuring temperature variations in the tissues. It detects pocket temperature differences of 0.1°C from a referenced subgingival temperature. This probe can detect initial inflammatory changes so that treatment can be initiated at an early stage.

4. Advances in microbial analysis

4.1. Bacterial culturing
Bacterial culturing is considered as a gold standard method as they are highly efficient. There are two types of culture methods - aerobic and anaerobic. The samples are cultivated under anaerobic condition because majority of periodontal pathogens are anaerobes in nature. Various anaerobic chambers include biobags, prereduced anaerobically sterilized media, anaerobic chambers and anaerobic jars that can be used with a combination of basal and selective media for the isolation of periodontal pathogens.

4.2. DNA probe
DNA probes work on the principle of the DNA base sequencing. DNA molecules are denatured or split into 2 separate strands. This is done by treating the plaque specimen with a detergent to lyse the cells and then boiled in a high pH solution. The denatured DNA is immobilized on nitrocellular filter. The filters are prehybridized with either EDTA or 0.5% Sodium dodecylsulfate for 1 hour at 65°C. The radiolabelled nucleic acid strands is then added to the immobilized specimen and hybridized in the same buffer by adding 10% dextran sulphate. This is then exposed to auto radiographic plate.

4.3. Omnipgene
They are used as DNA probe systems for a number of sub gingival bacteria.

4.4. Microprobe Corporation
It is designed as an in office nucleic acid probe assay for the semi quantitative detection of periodontal pathogens.

4.5. Perio 2000
This system is designed to display the sulphide level digitally produced by pathogens.
4.6. Polymerase chain reaction (PCR)
In the year 1990, PCR was introduced by Mullis and he won Nobel Prize for this discovery. It amplify DNA sequences by a method which involves using short DNA sequences to select the portion of genome to be amplified. The PCR test was able to identify periodontal pathogens like Porphyromonas gingivalis.

4.7. Chromatography
(Figure – 7) In this process, mixture to be separated is applied on a stationary phase (solid or liquid) and a pure solvent such as water or any gas is allowed to move slowly over the stationary phase, carrying the components separately as per their solubility in the pure solvent. The factors effective on this separation process include molecular characteristics related to adsorption, partition, and affinity or differences among their molecular weight. Because of these differences, some components of the mixture stay longer in the stationary phase, and while others pass rapidly into mobile phase, and leave the system faster.

![Figure 7 Chromatography](image)

4.8. BANA test (N-benzoyl-DL-arginine-2-naphthylamide)
BANA test is a rapid chairside diagnostic test which can give information about the bacteria by hydrolyzing trypsin-like enzyme. During hydrolysis it releases chromophore naphthylamide and when a drop of garnet is added to the solution it turns into orange-red. Weak positive result shows small faint trace of blue coloration on a pale red-brown background. Positive result shows patches of blue coloration larger and darker in size than weak positive result. Negative results show no blue coloration.

5. Advances in immunodiagnostic techniques

5.1. Immunofluorescence (IF) Assay
It is a histochemical laboratory staining technique that relies on antibodies– antigens interactions in tissue or body fluids.

Direct immunofluorescence is a technique where fluorescently labelled antibody specific to the target antigen is used in a patient tissue or cell whereas indirect immunofluorescence is usually used to detect specific patient antibodies.

Indirect immunofluorescence complement fixation is more sensitive than indirect IF because it uses amplification principle. The generation of antigen–antibody complexes activate the complement system to release C3 molecules that can be detected by anti-C3 and double immunofluorescence allows the detection of two different antibodies on cells by using two specific antibodies for the target antigens.

5.2. Radioimmunoassay
This technique achieves sensitivity through the use of radionuclide and specificity that is uniquely associated with immunochemical reactions. The first immunoassay developed was described by Yalow and Berso in 1959. They used
radio labelled insulin to assess the concentration of insulin in human plasma and thus developed the first radioimmunoassay (RIA).

5.3. Linked Immunosorbent Assay

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are both used as analytical tools in biomedical research for the detection and quantification of specific antigens or antibodies in a given sample. These two procedures share similar basic principles and are derived from the radioimmunoassay (RIA). RIA were modified by replacing the radioisotope with an enzyme and thus creating the EIA and ELISA.

Types - indirect, sandwich, competitive and multiple and portable ELISA (figure - 8).

![ELISA technique](image)

**Figure 8** ELISA technique

5.4. Flow cytometry

(Figure - 9) Flow cytometry is an instrument which can measure multiple physical characteristics of a single cell such as size and granularity as the cell flows in suspension through a measuring device. It depends on the light scattering features of the cells under investigation, which may be derived from dyes or monoclonal antibodies. Flow cytometry has the ability to measure the optical and fluorescence characteristics of a single cell or any other particle such as microorganisms, nuclei and chromosome preparations in a fluid stream when they pass through a light source.

![Flow cytometry](image)

**Figure 9** Flow cytometry

5.5. Latex agglutination test

(Figure - 10) Agglutination is a reaction of clumping together of antigen bearing cells, microorganisms or particles in the presence of specific antibodies (agglutinins) in a suspension. Reaction time is shorter as compared to other antigen-antibody interactions. It makes use of latex particles which are built from different organic materials to a desired diameter, and can be functionalized with chemical groups to facilitate attachment of molecules. The use of latex beads
was first described for the detection of rheumatoid factor by Plotz and Singer in 1956. Since then, latex tests have been developed to detect specific infectious diseases, autoimmune diseases, hormones, drugs, and serum proteins.

Figure 10 Latex agglutination test

6. Advances in assay based on molecular biology techniques

6.1. Nucleic Acid Hybridisation

When a double stranded molecule of deoxyribonucleic acid is heated, the complementary strands will separate the hydrogen bonds and interactions between the base breaks. When these single strands are cooled, they will reunite to form a double stranded molecule identical to the original DNA duplex. This reannealing or reunion of the single-stranded DNA is referred to as nucleic acid hybridisation and occurs because the single strands have complementary sequences. When this technique is applied to separate DNA strands from two different cells, the extent of similarity between the base sequences of the two DNA strands are determined. The greater the degree of hybridisation, the closer is the similarity. The purpose of the technique is to identify or localize certain nucleic acid sequences in the genome of species.

When hybridization takes place on a solid carrier it is named as blotting and is divided in 3 categories: Southern blotting where DNA molecules are identified using DNA or RNA probes,

Northern blotting where RNA molecules are identified using RNA or DNA probes,

Western blotting whereby protein sequences are identified using specific antibodies.

6.2. Next Generation Sequencing

It is also termed as massively parallel sequencing. This is capable of precise, fast and high genome sequencing and involves a new age in DNA diagnostics. This technology is capable of sequencing over 600 giga bases at more than 98% accuracy at a time. The advantage over traditional sequencing is that very large amounts of data is possible from highly complex heterogeneous mixes of template. It means whole genomes can be sequenced from one reaction.

6.3. Nucleic acid amplification

The amplification generates a large number of target copies, greatly enhancing the assay sensitivity. The most widely used amplification procedures include the polymerase chain reaction (PCR) which is based on a polymerase activity for primer directed target amplification. Advantages of PCR amplification is its simplicity and is cost-effective. It also has the potential for a single DNA molecule amplification due to its highly efficient exponential process but it also has drawbacks like it has time consuming thermal cycling process.

These drawback of PCR lead to development of nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), rolling circle amplification (RCA), multiple displacement amplification (MDA) and recombinase polymerase amplification (RPA).
6.4. Nucleic acid sequencing

DNA sequencing is the experimental method to determine the sequential arrangement of nucleic acid bases like A, T, G, and C in a polynucleotide which encodes different proteins in a living cell. Various applications of nucleic acid sequencing includes structural genomics, functional genomics, epigenomics, and metagenomics.

7. Advances in identifying biomarkers

7.1. Oral fluid nanosensor test

This device was developed by the University of California, Los Angeles Collaborative Oral Fluid Diagnostic Research Laboratory, by Dr. David Wong to detect oral cancer in saliva. This is an automated point of care device that is designed for the electrochemical detection of multiple salivary proteins and nucleic acids. Four salivary mRNA biomarkers (SAT, ODZ, IL-8 and IL-1β) and two salivary proteomic biomarkers (thioredoxin and IL-8) are detected in this system.

7.2. Electronic taste chips

This lab-on-a-chip system was developed at Rice University in Houston, Texas, which will differentiate between healthy and periodontally diseased individuals based on the CRP levels. This microchip based detection system is used for measuring analytes in solution phase. This novel system is called an electronic taste chip (ETC).

7.3. OraQuick

This test is done to diagnose HIV infection and it provides rapid results in 20 minutes. This test uses oral fluid to check for antibodies to HIV Type 1 and HIV Type 2.

7.4. Integrated microfluidic platform for oral diagnostics

This test helps in the rapid quantification of salivary biomarkers related to oral disease. It facilitates analysis by integrating pretreatment sample with electrophoretic immunoassays in a short period of time. Rapid measurement of levels of MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved in 3-10 minutes.

7.5. Microbiological test - MyPerioPath

It detects the pathogens causing periodontal disease in saliva samples. This test uses DNA polymerase chain reaction to detect the type and concentration of bacteria present in the salivary sample. The patient has to rinse with saline for 30 seconds then spit in a collection tube. The samples are then sent to the laboratory for microbiological analysis.

7.6. Omnigene

They are species specific DNA probes to identify eight pathogens which are known to cause periodontal disease like Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Eikenella corrodens, Campylobacter rectus, Bacteroides forsythus and Treponema denticola. The advantage of using these test kits is that the results can be obtained in short period of time and can be mailed or faxed to the clinician. This test detects microorganisms by using RNA probes.

7.7. Genetic test – MyperioID

It identifies the genetic susceptibility of the patient to periodontal diseases by using salivary samples which are shipped to the laboratory for results.

7.8. Periogard

It is based on the detection of an enzyme called aspartate aminotransferase (AST). AST is a soluble intracellular cytoplasmic enzyme that is released from the cell when it dies. AST levels in GCF are markers of early periodontal tissue destruction as cell death occurs during periodontitis. Increase in AST levels in a 30 second sample indicates an active diseased site.

7.9. Periocheck

It is the most rapid chairside test for detecting neutral proteases in GCF like elastases, proteinases and collagenase. But it has certain drawbacks like interproximal sites cannot be sampled due to saliva contamination, this test is not specific for collagenase and may include enzymes of bacterial origin.
7.10. Prognostik
This was developed in the year 1993; it measures the levels of elastase in the GCF. Active disease sites produce an elevated elastase level released from the lysosomes of polymorphonuclear leucocytes.

7.11. Matrix metalloproteinase dipstick test
MMPs are host-derived proteinases which plays a major role in periodontitis and peri-implant disease. This test will help in the rapid detection of pathologically elevated levels of MMP-8 in oral fluids and serum. Monoclonal antibodies for MMP-8 are being utilized in chairside point of care immunotests.

7.12. Perioscan (BANA)
P. gingivalis, T. denticola, T. forsythia and some Capnocytophaga strains produce bacterial trypsin like proteases in the dental plaque can be detected by this device. The enzyme produced by these bacteria hydrolyzes the synthetic peptide Benzoyl DL-arginine2-naphthylamide or BANA, present in the test.

7.13. Evalusite
It is a diagnostic kit based on the principle of ELISA. It involves the linkage between the antigen and a membrane bound antibody to form an immune complex. Putative periodontopathogens like bacterias of red complex can be detected using membrane based enzyme immunoassay.

7.14. Toxicity prescreening assay
It is a chairside test kit to detect bacterial toxins and bacterial proteins which are one of the markers of gingival infection. The principle behind this test relies on the detection of actively dividing and growing pathogens which can be accessed through the metabolic activity of these organisms in the crevicular fluid.

7.15. Genetic test - The periodontitis susceptibility trait test
It is the test which identifies the genetic predisposition of the patient for periodontitis by detecting the polymorphism in IL-1 gene. Polymorphism is seen in two positions of IL-1 i.e. position -889 and + 3953 and this shows that the site is associated with periodontal disease.

7.16. Advances in point of care diagnostic lab-on-chip method
A newer generation of point of care technology called lab-on-a-chip is under the process of development. This is basically a device which integrates and automates all the complexities of a laboratory procedure into a chip of a size of computer chip. This technology seeks to measure multiple biomarkers in a small saliva sample.

8. Advances in genetic testing

8.1. Family studies
Familial aggregation of severe non-syndromic aggressive periodontitis is a common finding. Segregation analysis and linkage analysis indicates that there are multiple different genetic forms of aggressive periodontitis, but the number of genes involved remained unclear.

8.2. Twin studies
Studying phenotypic characteristics of monozygotic twins is a method of differentiating variations due to environmental and genetic factors. Monozygotic twins are genetically identical and of same gender. Therefore, discordance in disease between twins must be due to environmental factors, but discordance between dizygotic twins could arise from both environmental and genetic variance.

8.3. Population studies
Environmental or behavioral risk factors for a disease were first detected in large epidemiological or population based studies. The frequencies of polymorphisms of candidate genes can be compared between cases and controls.
9. Conclusion
For the clinician, the planning of therapy is the most critical and difficult step in the treatment of patients with periodontal disease. A new paradigm for periodontal diagnosis will increase the understanding of periodontal diseases, which will increase the predictability of treatment and improve the clinical management of patients with periodontal disease.

With the progress being made in the field of technology and science there is an advancement that is observed in the understanding of the etiology and various the factors responsible for periodontal disease. This broader understanding is helping in the development of various newer diagnostic modalities in the field of Periodontology. However, there is much yet to be discovered to remove all the present obstacles and provide a better technology and materials for the future.

Compliance with ethical standards

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All authors declare no conflict of interest.

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