Advances in periodontal diagnosis

Periodontitis: is a dynamic disease process characterized by periods of disease progression, remission and exacerbation.

Diagnosis of periodontal disease includes different levels:

1. Diagnose periodontal health versus disease.
2. Classify the different types and severity of gingivitis and periodontitis.
3. Decide as whether the patient periodontitis is **active** or it is **arrested** or in **remission**.

In conventional diagnosis and classification of periodontal disease for a given patient primarily we depend on clinical assessment with many factors as

1. Presence or absence of clinically detectable inflammation.(gingivitis)
2. Extent and pattern of clinical attachment loss.(periodontitis)
3. Patient age at onset.
4. Rate of progression.
5. Presence or absence of miscellaneous signs and symptoms including pain, ulceration and amount of observable plaque and calculus.

To reach a good diagnosis we examine:

1. Gingival tissue in health and compare with signs of inflammation.(gingival index,bleeding on probing…..etc)
2. Loss of attachment assessed by probing pocket depth with calibrated instrument.(periodontal probes)
3. Radiographic evidence of alveolar bone loss.
4. Demographic data as: age, gender, etc…
5. Medical history and history of previous and current periodontal problem and miscellaneous clinical features or observation.

For most cases, the conventional diagnostic procedures are sufficient to design an effective treatment plan. But these methods have poor sensitivity in diagnosis sites or patients with active disease progression.

Therefore currently the scientists introduce other factors in the process of diagnosis as Risk and Risk factors which many predispose an individual to disease initiation and progression. Although many

diagnostic procedures hold promise only few are yet tried enough to be useful in the day-to-day practice of dentistry. **It is hoped that improved diagnosis of periodontal disease will better:**

- Differentiate between periodontal diseases.
- Identify persons and teeth that are susceptible to disease initiation and progression.
- Monitor the response to treatment.
- Identify disease initiation and progression.

In medical diagnosis, test sensitivity **is the ability of a** test **to correctly identify those with the disease (true positive rate), whereas** test specificity **is the ability of the** test **to correctly identify those without the disease (true negative rate).**

The sensitivity of a test (also called the true positive rate) is defined as the proportion of people **with** the disease who will have a **positive** result. In other words, a highly sensitive test is one that **correctly identifies patients with a disease**. A test that is 100% sensitive will identify *all* patients who have the disease. It's extremely rare that any clinical test is 100% sensitive. A test with 90% sensitivity will identify 90% of patients who have the disease, but will miss 10% of patients who have the disease.

The specificity of a test (also called the True Negative Rate) is the proportion of people without **the disease who will have a** negative **result. In other words, the** specificity **of a test refers to how well a test identifies patients who do not have a disease. A test that has 100% specificity will identify 100% of patients who do not have the disease. A test that is 90% specific will identify 90% of patients who do not have the disease and will miss 10% of patients who** do not **have the disease.**

Advances in clinical Diagnostic methods:

1--Gingival bleeding
It's an indicator of inflammatory **lesion** but its relation to disease activity is not clear yet. The normal force applied on probing is 0.25 Presence of positive bleeding on probing is not an indicator but negative bleeding or absence of bleeding indicates health.(this is in non smoker patients)

2--Probing technique:

Controlled – force, standardized probes:

The periodontal probe is the most widely used periodontal diagnostic tool for clinically assessing connective tissue destruction secondary to periodontitis.

Problems associated with conventional probe are:

Probing technique
Force.
Probe diameter and angulations.
Presence of inflammation

This has been solved with the development of pressure-sensitive probes, which have a standardized controlled insertion pressures.

Pressure sensitive probe: it has a standardized, controlled insertion pressure. It uses a fabricated stent for reproducibility

30 gm ⟶ tip with the junctional epithelium.

50 gm ⟶ periodontal osseous defect.

Recently automated periodontal probes and probing systems have been developed that allow the integration of direct electronic measurements at a constant probing force with computer storage and on-line data readout

I. **Automated Florida disc periodontal probe.** Which give a certainty of 99% detect of loss of attachment level of less than 1mm. it utilizes a reproducible Occlusal landmark or a customized stent margin as a reference land mark. Its probe hand piece connected to a monitor for digital read out and foot switch all to computer. It applied a constant force through a coil spring inside the probe.

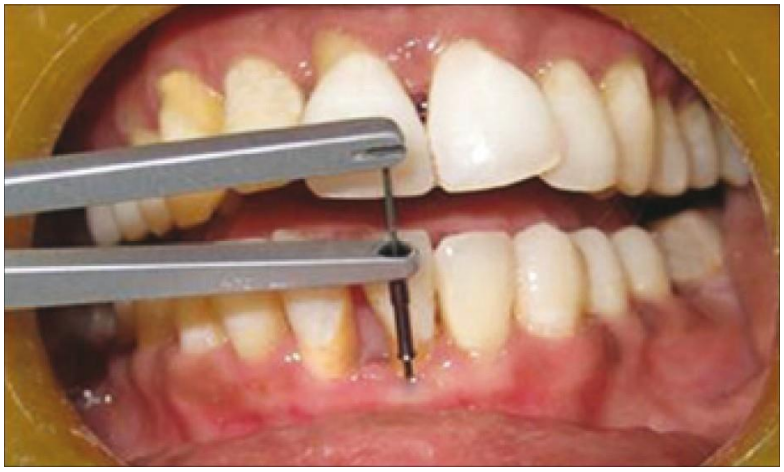
Advantage: it applied constant force and no need for assistant.

Disadvantage: lack of tactile sensitivity ,under estimation of deep

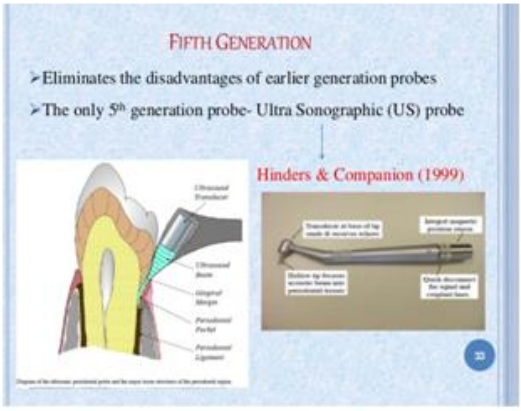
probing points and fixed probing force.

II. **Automated Foster-Miller probe:** it registers the C.E.J as its attachment level landmark. Some investigations reveal that this probe detect up to 0.2mm loss in attachment. It capable of coupling pocket depth with detection of C.E.J.

The **disadvantage** of such probe is time consuming**.**



Other electronic probes/inter probe ,perio probe,TorontoAutomatic



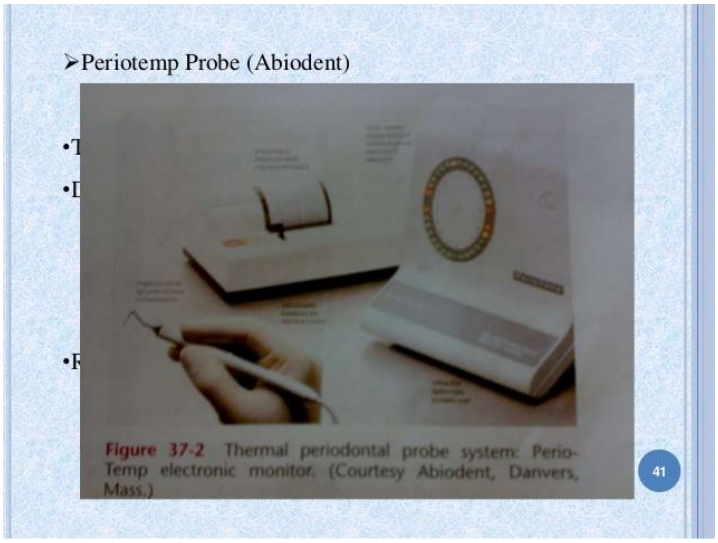
3--Indicators of local physical metabolic changes (gingival temperature)

Subgingival temperature: Like other signs of inflammation has good specificity but poor sensitivity when considered alone as a marker for progressive periodontitis.

It appears to be an anatomic temperature gradient in the oral cavity where mandibular periodontal sites are hotter than maxillary sites ,posterior wormer than anterior site. Also it appears that there is positive correlation between elevated subgingival temperature and:

- Severity of disease.(amount of attachment loss)
- Degree of subgingival inflammation.it increased at the diseased site due to increase in cellular and molecular activity
- Presence of putative pathogens.
- Smoking state .smokers have differences in sub gingival temperature and sub lingual temperature

Sub gingival probe is the tool used for measuring subgingival temperature. It is also called the perio temp. probe (Abiodent) which detect the difference in the temperature of 0.1c. The warm area signaled with red emitted



Advances in radiographic assessment

Conventional radiograph: it is a traditional method used to assess the destruction of alveolar bone. It is very specific but lacks sensitivity.

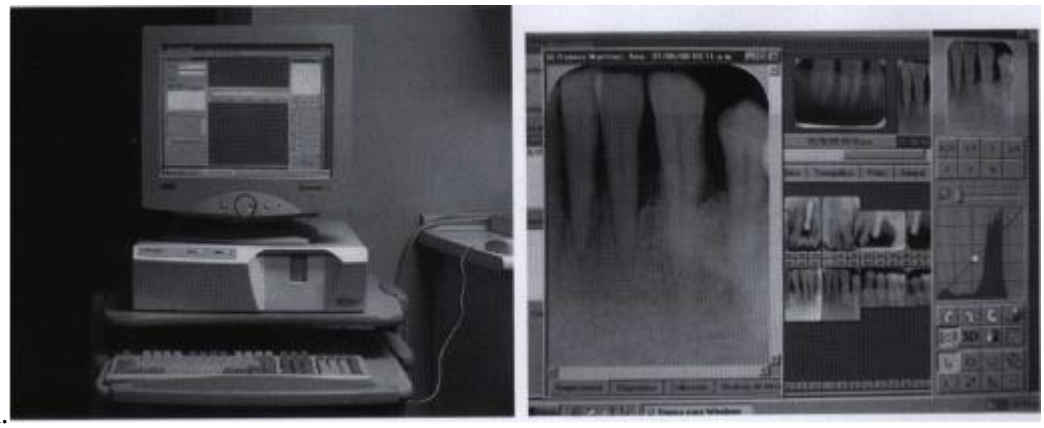
Problems associated with conventional radiograph

- Variations in the projection geometry.
- Variations in contrast and density due to differences in film processing, voltage and exposure time.
- Masking of osseous changes by other anatomic structures.

For standardization and reproducibility:

- Use film holder

- Use template with impression material.
- Extension arm to both film holder and X-ray.

Digital radiograph (D.R.): It enables the use of computerized image which can be stored and manipulated. 

Advantages: digital storage, image enhancement, and radiation dose reduction

Two D.R. systems: -Direct method -Indirect method

Direct method: used charged coupled device, sensor linked with fiber optic to computer system. It provides 1/3 -1/2 reduction in radiation dose.

Indirect method: used phosphor luminescence plate which is flexible, film like radiation energy sensor placed intraorally and exposed to conventional X-ray tube, a laser scanner read the exposed plate and produce digital image.

Subtraction radiography:

Conversion of serial radiographs into digital images. The image then superimposed and the resultant composite viewed on a video screen.

Bone gain ⟶ lighter area

Bone loss ⟶ darker area.

Limitations for this technique is the need to paralleling technique and accurate superimposition

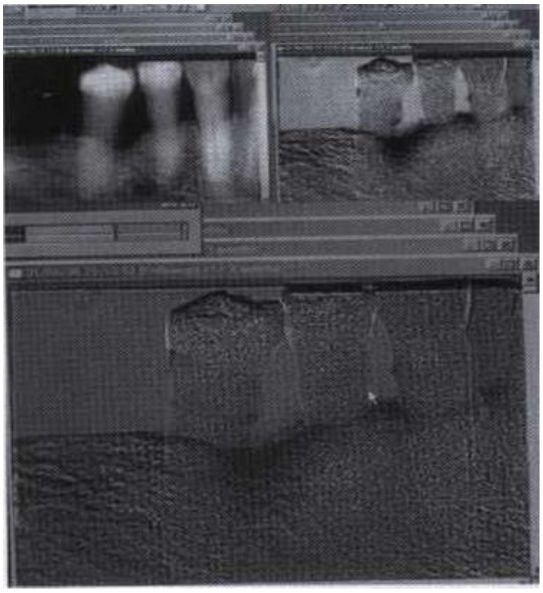
Advantages:

- Correlation between change in alveolar bone shown in subtraction radiograph and CAL change, post therapy.

- Increased detect ability of small osseous lesions.
- Both quantitative and qualitative visualization
- More sensitive

Disadvantages;

Identical projection alignment during sequential radiographs



Computer-assisted-densitometric-image-analysis (CADIA)

3D quantification of bone volume change. The device includes camera, image processor and computer. A video camera measures the light transmitted through a radiograph, and the signals from camera are converted into gray-scale image

Advantages

*This method increases accuracy, reproducibility and sensitivity.
This system appears to offer an objective method for following alveolar bone density changes quantitatively over time, and, when compared with digital and subtraction analysis, it has shown a higher sensitivity and a high degree of reproducibility and accuracy.

Cone Beam Computed Tomography (CBCT)

Utilizes a cone shaped source of radiation and an area detector and that it acquires a full volume of images in a single rotation with no need for patient movement. CBCT system accompanying software, any number of diagnostic images can be generated.

Advances in Microbiologic Analysis

Although over 300 bacterial species make up the oral flora. It is currently thought that only a few either alone or in combination initiate periodontitis progression. Strong evidence related to Aa, Pg,and Tf .Other m.o are thought to have etiologic role are;comphylobactor rectus, Euobacterium nodatum, Fusobacterium nucleatum and Prevotella intermedia and nigrescens

Uses of microbiologic analysis;
*support diagnosis
*aid in treatment planning
*good indicator of disease activity

Culture techniques have been the primary method of identifying putative pathogens. It allows:

- Characterizing subgingival flora (count (relative and absolute), and morphology).
- For speciation and antibiotic susceptibility testing.

Cultivation for plaque done under anaerobic condition using selective and non selective media together with several biochemical and physical tests, the different putative pathogens can be identified.

- **Limitation of cultures:**

1-Technical problems.

Culture methods can only grow live bacteria; therefore strict sampling and transport conditions are essential. Moreover, some of the putative pathogens, such as *Treponemas sp.* and *Bacteroides forsythus* are fastidious and difficult to culture.

2-Cultivating micro-organisms can be both time consuming and costs.

3-Low sensitivity .organisms lesser than 10^3in periodontal pocket is difficult to detect

Direct Microscopy:

Dark field or phase contrast microscopy has been suggested as an alternative to culture methods on the basis of its ability to directly and rapidly assess the morphology and motility of bacteria in a plaque sample. It has been used to indicate periodontal disease status and to structure maintenance programs. However, most of the main putative periodontopathogens, including *Actinobacillus actinomycetemcomitans, P. gingivalis, B. forsythus, Eikenella corrodens,* and *Eubacterium* species, are non-motile, and therefore this technique is unable to identify these species.

Molecular biology techniques:

The bases principle is to analysis DNA, RNA and the protein structure. Rely on species specific genomic sequence for microbial identification

NUCLEIC PROBES.

include two probes :DNA probe , and oligonucleotide probe.

Deoxyribonucleic Acid Probe Technology:

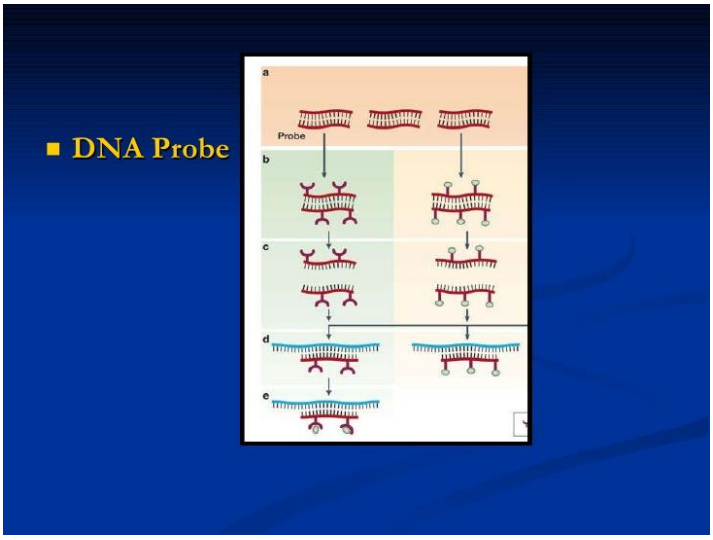
Deoxyribonucleic acid (DNA) probes entail segments of single-stranded nucleic acid, labeled with an enzyme or radioisotope that can locate and bind to their complementary nucleic acid sequences with low cross-reactivity to non target organisms. DNA probe may target whole genomic DNA or individual genes. Whole genomic probes are more likely to cross-react with non target microorganisms due to the presence of homologous sequences between different bacterial species. However, specific genes, such as some of RNA (ribonucleic acid) genes, contain signature sequences limited to organisms of the same species. To prepare the probe, specific pathogens used as marker organisms are lysed to remove their DNA. Their double helix is denatured, creating single strands that are individually labeled with a radioactive isotope. Subsequently, when a plaque sample is sent for analysis, it undergoes lyses and denaturation. Single strands are chemically treated, attached to a special filter paper, and then exposed to the DNA library.

Steps of DNA-analysis method

First: a labeled DNA (probe)s are constructed with a radioactive or enzyme detection system.

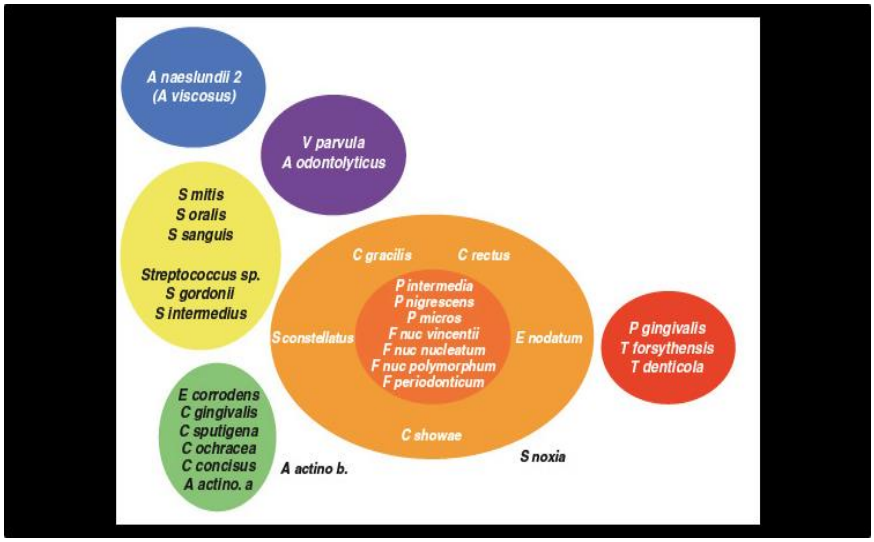
Second: sub gingival plaque are collected on a sterile paper points or dental curettes and enzymatic ally yielding single, stranded, denaturized DNA fragments.

Third: the unknown fragments are then exposed to complementary labeled probe and allowed to hybridize. (hybridization refers to the pairing of complementary DNA strands to produce a double – stranded nucleic acid)



Test response reflects both the presence and approximate number of tented pathogen up to 10^3. Most often the DNA probe can test p.gingivalis, prevottella intermedia, A.a, Eikenella corrodens, etc..

2-**checkboard DNA-DNA hybridization technology** is a recently established technique that gives a simultaneous and quantitative analysis of up to 28 plaque samples against 40 microbial species (Socransky 1994) .DNA checkboard method offers the ability to include more potential periodontal pathogens in large scale studies with a single analysis or test than is usually practicable with cultural analysis. With this method the associated pathogens that related to periodontitis are set in clusters known as plaque complex each color represents a cluster of associated periodontal pathogens. It is particularly applicable for epidemiologic research and ecologic studies because it does not require viable bacteria and allow for the assessment of large number of plaque samples and multiple species



3-polymerase chain reaction (PCR) this reaction is an in-vitro enzymatic reaction that involves the application of specific DNA sequences. It is the most powerful tool to amplify the genes and their RNA transcripts.

The bases of PCR is the use of DNA polymerase which is an enzyme that catalyzes the formation and repair of DNA and can make a copy of the entire DNA in each chromosome .PCR typically begins with the isolation of DNA from a fresh tissue specimen .By heating the complementary double strands, DNA splits into single stranded forms intended to act as the template dictating the nucleotide sequence in vitro.

Immunologic-based tests for putative pathogens (Immunodiagnostic Methods):

Immunologic assays employ antibodies that recognize specific bacterial antigens to detect target microorganisms. This reaction can be revealed using a variety of procedures, including direct and indirect immunofluorescent microscopy assays (IFA), flow cytometry, enzyme-linked immunosorbentg assay (ELISA), membrane assay, and latex agglutination.

immunofluorescent microscopy assays (IFA)

Detect sub gingival pathogens using monoclonal antibodies specific to bacterial antigens. One technique for this is **immunofluorescent microscopy** which can detect bacterial level as low as 10^4 using fluorometer and microscope. The immunofluorescence assay done in two ways: direct and indirect.

Direct method include the interaction between antibody(monoclonal and polyclonal antibodies) that is conjugated with fluorescence marker with bacterial cells to make an immune complex detectable under a microscope while the indirect method include the interaction between immune complex (primary antibody +bacterial cells) with secondary fluorescence conjugated antibody. Both direct and indirect

immunofluorescence assays are able to identify the pathogen and quantify the percentage of the pathogen directly from a plaque smear.

***Elisa test:** an enzymatic ally derived color reaction. It detects serum periodontal pathogens.

***flow cytometry**

A technique for identifying and sorting cells and their components (as DNA) by staining with a fluorescent dye and detecting the fluorescence usually by laser beam illumination
this sophisticated technique, measure properties of cells in a sample of bone marrow, lymph nodes, or blood. The sample is first treated with special antibodies and passed in front of a laser beam. If the antibodies attach to the cells, the cells will give off light. Looking for certain substances, or antigens, on the surface of cells helps us to identify the cell type.

*latex agglutination test which is simple ,rapid method highly sensitive and specific

Latex beads coated with species specific AB. when beads come in contact with specific species in the sample they bind and agglutination occurs lead to clumping of beads is visible so test is positive

Enzymatic Methods of Bacterial Identification:
which is a rapid method, screen for the presence of an enzyme unique to one or more species.
Ex; BANA hydrolysis test

Some of periodontopathogens share a common enzymatic profile, since all have in common a trypsin like enzyme. The activity of this enzyme can be measured with the hydrolysis of the colorless substrate N-benzoyl-dl-arginine-2-naphthylamide (BANA). When the hydrolysis takes place, it releases the chromophore ß naphthylamide, which turns orange red when a drop of fast garnet is added to the solution. Diagnostic kits has been developed using this reaction for the identification of this bacteria profile in plaque isolates (Perioscan)

The synthesis of trypsin-like enzyme mostly by 3 pathogens (p.gingivalis, B.forsythus and T.denticola). Perioscan is popular kit uses BANA test

<u>Advances in characterizing host response</u>

Asses host response by studying mediators as a response to specific bacteria or local release of inflammatory mediators or enzymes as in response to infection .source of the sample include blood serum ,saliva and GCF(gingival crevicular fluid),gingival crevicular cells ,blood cells and urine.

Assessment of the susceptible host using makers in peripheral blood:

I. **<u>Polymorph nuclear leukocytes (PMNs):</u>** measuring peripheral blood neutrophils functions (chemotaxis and phagocytosis) is a way to screen individuals at high risk for

active periodontal disease progression. It is done on a limited basis in research patient because it's ***expensive*** and ***time consuming***.

II. **Antibody titer:** circulating peripheral blood contains antibodies against periodontal pathogens. Investigators have tried to correlate elevated serum antibody level to specific bacteria with episodes of periodontal disease activity or tissue breakdown.

It's at present not clear whether elevated serum antibody titer is a sign of protection of an individual against pathogens in general and thus disease activity or whether this is a host response associated with active periodontal destructions.

Identification of host constituent in crevicular fluid

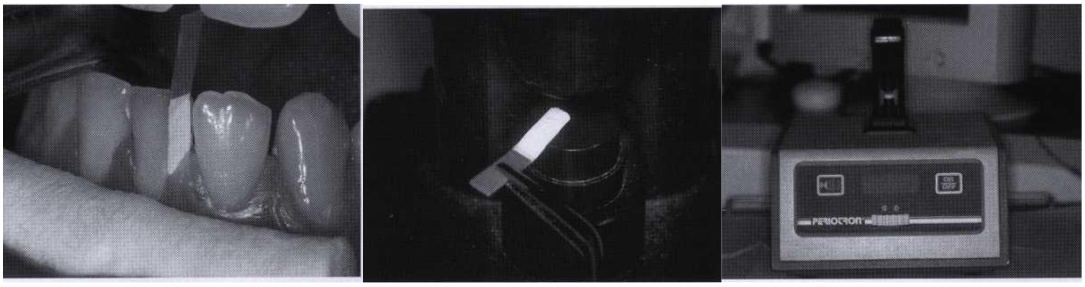
Gingival crevicular fluid (GCF) is a serum like exudates which bathes the gingival sulcus or periodontal pocket, and which follows an osmotic gradient with local tissue.
As this fluid traverse from the host microcirculation, through inflamed tissue, and into the periodontal pocket, it captures mediators involved in the destructive host response, and by products of local tissue metabolism.
These constituent in GCF may be harvested through:

- Filter paper or strips
- Capillary tube include micropapillary tube, micropipettes, microsyringes
- Intrasulcus washing

The most widely method used for collection of GCF is paper strips. These strips are placed in the gingival sulcus for a standard period of time until the filter paper is saturated, the fluid volume collected on the strips can be then quantified in a number of ways. At present the most popular way with specific assay is using the Periotron® device or test the differences in the weight of the paper between pre and post collection.

The periotron measure the capacitance across the wet paper strip, which convert to digital reading. Periotrone readings have high correlation with clinical gingival indices and its quickest and easiest way to measure GCF

From these diagnostic markers in GCF are: three main groups: host-derived enzymes, tissue breakdown products, and inflammatory mediators.



Concentration of these mediators in GCF from a disease sites may be higher than from a healthy sites.

1. Inflammatory Mediators and Products

Cytokines are potent local mediators of inflammation that are produced by a variety of cells. Cytokines that are present in GCF and have been investigated as potential diagnostic markers include tumor necrosis factor alpha (TNF- a), interleukin-1 alpha (IL-la), interleukin-1 beta (IL-1ß), interleukin-6 (IL-6), and interleukin 8 (IL-8).

IL-1, IL-6, and TNF-a are cytokines produced by a variety of cells at inflamed sites. They are potent immune regulatory molecules with a variety of biologic effects, including metalloproteinase stimulation and bone resorption; therefore they seem good candidates for markers of disease progression.

Prostaglandin E2 is a product of the cyclooxygenase pathway of the metabolism of arachidonic acid. It is a potent mediator of inflammation and induces bone resorption. In cases of untreated periodontitis, the concentration of prostaglandin E2 found in GCF increased during active phases of periodontal destruction.

2. Host-Derived Enzymes

Various enzymes are released from host cells during the initiation and progression of periodontal disease. The enzymes that have received the most attention as possible markers of active periodontal destruction are aspartate aminotransferase (AST), alkaline phosphatase, ß-glucuronidase, elastase, cathepsins, and matrix metalloproteinases. Some of these enzymes are released from dead and dying cells of the periodontium; some come from polymorph nuclear neutrophils; and others are produced by inflammatory, epithelial, and connective tissue cells at affected sites.

3. Tissue Breakdown Products

One of the major features of periodontitis is the destruction of collagen and extracellular matrices. Analysis of GCF obtained from sites with periodontitis clearly shows elevated levels of hydroxyproline

from collagen breakdown and glycosaminoglycans from matrix degradation. Other bone and connective tissue proteins, including osteocalcin and type 1 collagen peptides, have been correlated with the progression of alveolar bone loss induced in beagle dogs. Both markers gave high positive predictive values and now need to be extended to longitudinal studies in humans.

Markers in saliva

Saliva is a transudation from serum. Secreted from oral salivary glands. It provides an easy method for collection which it is either *stimulated* or *unstimulated* method. It also provides method for collection of large sample for estimation.

Flow rate of saliva and salivary pH can be estimated and correlate with some systemic conditions. Saliva may contain locally and systemically derived markers of periodontal disease which include:

- Proteins.and enzymes of host origin
- Hormones (cortisol). Phenotypic markers and host cells.
- Bacteria and bacterial products and volatile compounds
- Ions ,Micro and macro elements.