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Review Article

An update in advanced diagnostic aids in periodontal diagnosis – A review

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ABSTRACT

Periodontitis is a chronic infectious disease leading to destruction of periodontal structures, if untreated leads to tooth loss. An correct diagnosis is based on a comprehensive examination. The development of diagnostic techniques allows for the assessment of a patient's reaction to periodontal therapy, the determination of whether the disease is active, and the prediction of the illness's future course. This review article discuss the recent updates in the diagnostic tools for periodontal diagnosis.

1. Introduction

Periodontitis is a chronic immuno-inflammatory condition creating progressive destruction of periodontal tissues (alveolar bone, periodontal ligament, and cementum) leading to tooth loss. An accurate diagnosis is essential for managing periodontal diseases. It is based on comprehensive clinical examination, radiographic, histological examinations, patient's medical & dental records.[1] Traditional diagnosing clinical markers like bleeding on probing, probing pocket depth, clinical attachment loss, and the periodontal index, are of limited use because they indicate past periodontal issues rather than current disease activity.[2] On the flip side, novel diagnostic methods enhance the clinical management of individuals with periodontal disease. These techniques not only identify the current presence of the disease but also predict its future development and evaluate the effectiveness of periodontal therapy. Periodontal diagnostic help in monitoring the progress of therapy, identify sites or individuals at a higher risk of disease progression, screen for active disease, ensure accurate diagnosis, establish prognosis, and guide treatment plans.[3] Traditional methods have certain limitations. They are unable to differentiate between positive & negative treatment outcomes, do not provide information about the patient's susceptibility to the disease, fail to offer a rationale for the condition and unable to precisely identify ongoing periodontal damage or sites that are in remission

2. Advanced Periodontal Diagnostic Techniques

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2.1. Advancements in clinical diagnosis

2.1.1. Evaluation of periodontal attachment loss

Periodontal probing is the primary diagnostic technique for determining pocket depth, involving measuring from the marginal gingiva to the depth of the pocket. Ongoing assessment of clinical attachment level or probing depth is considered the benchmark for documenting changes in periodontal status.

2.1.1.1. Classification of periodontal probes based on generation. First-generation probes - Williams periodontal probe, CPITN probe, and UNC-15 probe, are commonly used in dental examinations.[4] Limitations include, they often lack sensitivity and reproducibility, making it challenging to obtain accurate measurements. Additionally, these probes do not allow for control of probing force or pressure, leading to discrepancies in measurements. Inter-examiner differences, influenced by probing technique, force, angle of insertion, probe size, calibration precision, and the presence of inflammation, can further impact the accuracy of measurements taken with these probes. Second-generation probes, known as constant force probes or pressure-sensitive probes, aim to enhance measurement standardization by reducing variations in probing force. For example, a pressure-sensitive probe might be designed to exert a force of 30g when probing a pocket and 50g when probing an osseous defect. Pro-DenRxr Sensor Probe - without electronic control, this manually operated probe maintains a constant probing force. In 1980, Polson introduced an electronic pressure-sensitive probe that used audio signals to maintain consistent control at a rate of 0.25N. Originally called the Vine Valley Probe (Vine Valley Research, NY, USA), this tool has evolved into the Yeaple instrument, now utilized for dentinal hypersensitivity testing.[5] The current methods are limited by inaccuracies in data readout, the lack of an automated system for storing data, measurement discrepancies due to differences between examiners, and the laborious process of manually recording and analyzing extensive data collected from multiple sites. Third-generation probes, also referred to as automated probes, offer more than just consistent pressure application; they can also store data. This functionality aids in reducing examiner bias and enhancing precision. The Toronto probe, Florida probe, and Foster Miller probe employ computer-assisted direct data collection to achieve this goal.[4]The Alabama probe, developed by Foster Miller and described by Jeffcoat in 1986, provides controlled probe pressure and identifies the cementoenamel junction during pocket depth measurement. This is accomplished by manipulating the probe's ball tip along the root surface, with the tip's velocity adjusting as it nears the CEJ. It allows the probe to reach the base of the periodontal pocket until the desired force is exerted. Electronic means can estimate and monitor the clinical attachment level based on control speed, set force, slide times, and acceleration time history.[6]The Florida probe, developed by Gibbs et al. in 1988, features a hemispheric probe tip measuring 0.4mm. Its sleeve-edge provides a reference for making measurements. The probe is powered continuously by a coil spring and data is stored using a computer. The probe system provides a constant pressure of -15 grams and a precision of 0.2 mm. The Florida probe has three variants: the pocket probe, the disk probe, and a stent-based model.[5]The Florida probe has certain limitations. It doesn't offer the same level of tactile sensitivity as manual probing, which can affect its ability to provide feedback on tissue texture or firmness. Additionally, its fixed probing force may not be suitable for all cases and could lead to an underestimation of pocket depth in some instances. Furthermore, the probe's design and pressure application may result in an underestimation of the depth of deep periodontal pockets.

2.1.1.2. Toronto automated probe. McCulloch and Birek, in 1991 at the University of Toronto, used a probe on the occlusal/incisal surface to measure gingival attachment levels. This probe was equipped with a tilt sensor device in its handle to detect changes in probe angulation. Sulcus probing was performed using a 0.5-mm nickel-titanium wire extended under air pressure. An electric torque motor contained in the length gauge could generate probing forces of 0.1 N to 0.9 N, corresponding to probing pressures of 0.51 N/mm^2 to 4.58 N/mm^2.[7]The probe offers several advantages, such as an integrated electronics guidance system that enhances the accuracy of probe analysis. However, it has some disadvantages, including the potential for errors in probe positioning and challenges in recording pocket depths around second and third molars, as well as reproducing readings. Innovative tools in periodontal probing technology, such as the InterProbe and the Pa-on Parometer are making strides in the field. The Pa-on Parometer features a flexible probe tip designed to reduce probing pain. Both devices offer graphical and audible feedback, enhancing their usability with their ergonomic wireless design. These sophisticated electronic probing systems effectively mitigate inaccuracies in data capture and recording, ultimately streamlining the process of periodontal probing measurements.[8]A constraint of electronic probes is the absence of significant differences in accuracies or measurement variability compared to manual probes when assessing probing depth (PD) or clinical attachment level (CAL). It's also recommended to maintain a clear distinction between electronic and manual probes for measurement purposes. Additionally, additional training may be needed for the use of electronic probes, and their clinical use may be limited by costs. Fourth-generation probes, referred to as three-dimensional probes, are crafted to track the sequence of probe positions along the gingival sulcus base. They aim to advance linear probing by creating a continuous, three-dimensional map of the pocket

under examination. The development of these probes is ongoing.[9–11]

Fifth generation probe: Hinders et al. introduced 3D non-invasive probes in 1999. These instruments use ultrasound waves in addition to 3D technology. They are non-invasive probes, meaning they do not require penetration into tissues. The use of ultrasound waves is beneficial for accurately measuring attachment levels without directly touching the junctional epithelium.[4]

2.1.1.3. New advances in probes. In the field of force control, digitization, and automation, only a limited number of probes have been accepted in clinics due to their complex operation, poor cost-effectiveness, or lack of substantial improvements in accuracy and reproducibility. There is a need for more innovation to enhance the accuracy and reproducibility of diagnostics. For example, the development of multifunctional probes using nanotechnology and microfabrication for probe design shows promise in addressing these challenges.[10]

2.1.2. Assessment of tooth mobility

Microperiodontometer (Korber K.H 1963)[12]Mobility of teeth with non-working interferences can be detected with a microperiodontometer. A force of 500g was applied 3 times on the facial and lingual displacements were recorded

Piezoelectric transducer (Oka H., 1998)[13]A device that utilizes the piezoelectric effect to detect and measure changes in acceleration, pressure, strain, temperature, or force by converting these mechanical energies into an electrical charge.

Dental holographic interferometry (Wedendal P.R.,1974)[14]This is a non-contact and non-destructive method that utilizes a Q-switched double-pulsed ruby laser. This technique provides detailed information and its documentation is enhanced by a special photographic method

Non-contact vibration device, as described by Yamane M. in 2008, is an electromagnetic vibrating device that utilizes an alternating sine wave to generate force without physically contacting the tooth.[15]

Resonance frequency analysis (RFA) (Meredith in 1994)[16]A test to assess implant stability by measuring the frequency of implant oscillation inside the bone.

Laser vibrometer (Castellini P.,1999)[17]A small hammer and a load cell were utilized to apply dynamic loads on teeth, measuring the forces. Subsequently, a Laser Doppler vibrometer was used to measure tooth displacement. This method is easy to use and enables versatile non-contact measurements with high accuracy and sensitivity (< 0.1 mm/sec).

Periotest®M**,** developed by Medizintechnik Gulden in Modautal, Germany, is an electronic wireless device utilized for evaluating tooth mobility and the osseointegration of implants. Its functioning involves measuring the response to a consistent impact applied to the tooth surface's center. The device calculates the duration of contact per impact by employing an electrically controlled rod that strikes the tooth and then recoils. Variations in this contact duration may suggest structural alterations in the periodontal bone or soft tissue as a result of periodontal disease.[18]

Implomates, created by Bio Tech One Inc. in Taipei, Taiwan, is a device that employs an electromagnetic field to drive a metal rod for percussing the tooth. The device records the vibrations produced using a microphone with a resolution of 50 Hz.[19]

Osstell IDX- The Osstell IDX, developed by Osstell AB in Gothenburg, Sweden, is a non-contact device designed for measuring implant stability (osseointegration) through OSTELL employs Resonance Frequency Analysis (RFA) with resonance frequencies between 3000 and 8500 Hz to determine the implant stability factor on a scale of 0 to 100. It is a non-invasive and non-destructive method, consisting of a transducer, a computer analysis unit, and an excitation source.[20] Designed to enhance patient comfort and reproducibility in measuring implant stability, although it is optimized for assessing osseointegration rather than traditional tooth mobility. To minimize variations arising from test position and device operating conditions, tooth mobility tests using a non-contact vibration technique should be conducted.[21]

2.1.3. Examination of plaque and calculus

DetecTar probe is designed to detect subgingival calculus by analyzing optical signals on root surfaces and distinguishing spectro-optical variances between calculus and tooth surfaces. Subgingival calculus produces a unique spectral signature when exposed to a specific light wavelength, attributed to absorption, reflection, and diffraction. These signals are captured by a fiber optic and converted into an electric signal for computer analysis. The DetecTar probe tip is similar in shape and dimension (0.45 mm diameter) to conventional periodontal probes. Additionally, the system can serve as a portable cordless hand-piece with a curved periodontal probe that includes millimeter scale markings for measuring clinical attachment level and probing depth.[4]

Perioscopy, developed by Zest Dental Solutions in Carlsbad, CA, USA, involves using a miniature periodontal endoscope to visualize the root surface within the periodontal pocket. This technique provides magnifications of up to 48×, assisting in the identification of residual calculus spots during examinations.

Diagnodent**,** is a pen-like probe that emits a harmless, painless laser beam onto the tooth to detect autofluorescent signs of calculus lesions. Uses a range of values for relative calculus detection to measure fluorescence intensities, which are then shown on an electronic screen.[2]

PerioScan is capable of identifying calculus deposits and offers a treatment mode for traditional ultrasonic

debridement with adjustable power levels. It concurrently shows blue light and emits an acoustic signal on both the handpiece and screen to aid in diagnosis when the ultrasonic tip detects calculus on the tooth surface. Conversely, Key Laser 3 is designed for calculus detection and removal in a feedback-controlled manner. This automated device features a 655-nm In:Ga:As diode laser for calculus detection and a 2940-nm solid-state erbium-doped yttrium aluminum garnet (Er:YAG) laser for calculus removal. Some studies suggest that there are no statistically significant differences between feedback-controlled laser debridement and ultrasonic treatment. The Perioscan and Key Laser offer diagnostic and treatment modes that can be used continuously on the same tooth surface without changing instruments. However, these devices have limitations. Improvements are needed in the specificity of calculus detection, particularly in reducing false detections, where irregularities on the root surface are incorrectly identified as calculus. Sensor-integrated probes have been used to measure parameters not typically assessed in clinical examinations, such as temperature and sulfide concentration.[22]

2.1.4. Evaluation of gingival inflammation

2.1.4.1. Gingival temperature. Compared to normal healthy sites, subgingival temperature is elevated in diseased sites. In gingival sulci affected by periodontal disease, an increase in temperature ranging from 0.7 to 3.0°C has been observed compared to healthy sites. This temperature elevation is attributed to increased probing depth, which leads to heightened cellular and molecular activity caused by increased periodontal inflammation. The PerioTemp probe (Periotemp, ABIODENT, Inc, Danvers, MA, USA) allows for the measurement of temperature differentials (with a sensitivity of 0.1°C) between the probed pocket and subgingival temperature. Haffajee et al. 1992 discovered that sites displaying a red (higher) temperature indication had more than twice the risk for future attachment loss compared to those with a green indication. Elevated subgingival site temperature is also associated with attachment loss in shallow pockets and increased proportions of certain bacteria.[23,24]The PerioTemp probe offers several key benefits, including its rapid response time (<1 second), high accuracy (±0.1°C), and high reproducibility. It enables the measurement of clinical attachment level, probing depth, bleeding on probing, and temperature. The probe includes a computerized thermometer that shows the actual subgingival temperature and uses two-color light indicators to indicate risk levels.[25] The Thermoscan, renowned for its accuracy, has a mean difference of approximately 0.18°C for measured gingival temperature. However, using subgingival temperature changes as a method to evaluate periodontal disease is not reliable, despite its common use in clinical studies to assess disease progress and activity. This unreliability is due to significant differences among patients, examination site locations, and surrounding environmental factors (such as ambient temperature and respiratory airflow). Further research is necessary to enhance the consistency and reliability of subgingival temperature measurements before they can be considered a diagnostic tool.[2]

2.1.5. Measurement of volatile sulfur components

Periodontal diseases, particularly plaque-induced gingivitis, are linked to sulfide byproducts. Various tools are available for detecting sulfides, including the Halimeter, the Oral Chroma and the Breathtron. These tools are designed to detect halitosis and are not used for the diagnosis of periodontal disease. The Diamond Probe/Perio 2000 System is designed to offer real-time monitoring of gram-negative bacteria and sulfide levels in the gingival sulcus and periodontal pockets. This system utilizes a microscale sensor integrated into modified Michigan O sulfide periodontal probes to measure clinical attachment level (CAL), probing depth (PD), bleeding on probing (BOP), and sulfide levels. Upon detection of sulfides in the gingival crevicular fluid (GCF) by the sensor-integrated probe tip, the system provides information using a four-color light bar, an audible tone, and the actual sulfide level.

2.2. Progress in radiographic assessment

Dental radiographs are a conventional way to evaluate the degree to which alveolar bone has been destroyed by periodontitis. used to assess bone loss in angular patterns, such as intrabony defects, root morphologies, radiolucencies at the furcation, endodontic lesions, endodontic misshapes, developmental abnormalities, and the length and shape of the residual bone.

2.2.1. Conventional radiographs

From CEJ to the crest of alveolars, special but weak sensitivity and bone loss can be estimated.

2.2.2. Limitations

1. Change in the projection geometry.
2. Differences in the contrast and density between them.
3. Masked by another anatomic structure.

Subtraction radiography[26]Used to enhance the diagnostic information obtained from radiographs by removing superimposed images that are not useful for diagnosis. It provides a quantitative and qualitative view of minor variations in bone density. Here, two radiographic images are taken, one before and one after a certain treatment or time interval. The images are then digitally subtracted from each other, highlighting any changes that have occurred between the two images. In the resulting image, areas of

bone loss appear darker, while areas of bone growth appear lighter, allowing for a more precise detection of changes in bone density.

Digital radiography: Using a sensor to capture radiographic images revolutionized dental imaging. Trophy introduced the first digital radiographic images of teeth through the RVG Radiographic Imaging System in 1987.[27] This innovation brought several advantages: a) Elimination of chemical processing: Digital radiography eliminates the need for traditional film processing chemicals, reducing environmental impact and costs. b) Increased efficiency and speed: Digital images can be viewed immediately after capture, allowing for quicker diagnosis and treatment planning. c) Improved diagnostics: Digital radiographs can be enhanced and manipulated to improve diagnostic accuracy, such as adjusting brightness and contrast or zooming in on specific areas of interest. d) Computerized storage: Digital radiographs can be stored electronically, saving physical storage space and enabling easy retrieval for comparison over time or for referral. e) Reduced radiation exposure: Digital radiography typically requires less radiation exposure compared to traditional film radiography, enhancing patient safety.

Computer-Assisted Densitometric Image Analysis (CADIA) is a radiographic method introduced by Urs Bragger et al in 1988. This technique allows for the quantification of changes by measuring the radiographic density in a predetermined region between baseline and subsequent subtraction radiographs. The camera used in CADIA measures the light passing through the radiograph, and the signal captured by the camera is converted into a grayscale image. Advantages of CADIA include its ability to measure the quantitative change of bone density and its higher sensitivity, reproducibility, and accuracy compared to subtraction imaging.

Computed Tomography (CT) is a specialized radiographic technique that enables the visualization of specific planes or slices of interest. CT offers several advantages over conventional radiography, including the elimination of superimposition of images of structures in superficial or deep areas, the ability to distinguish differences between tissues of varying physical density due to its high contrast resolution, and the capability for multiplanar imaging, allowing multiple scans of a patient to be interpreted as images in axial, coronal, and sagittal planes, depending on the diagnostic requirements. However, CT also has some disadvantages. It requires specialized equipment and setting, involves higher radiation exposure compared to conventional radiography, and may produce ring artifacts from metallic restorations that can affect image diagnostics.

Cone-beam Computed Tomography (CBCT): A newer technology for acquiring 3D images of oral structures. It is cheaper than CT, less bulky, and produces a smaller dose of X-radiation.[26]

2.2.3. Advantages

1. Creates a complete 3D reconstruction.
2. Allows limited X-radiation exposure through beam collimation.
3. Reduces image artifacts.

Computer-Based Thermal Imaging: Compares the rewarming rate of normal and inflamed human gingiva to aid in the measurement of gingival temperature. It is a noninvasive method provided by infrared thermography.

Tuned Aperture Computed Radiography: Tuned aperture computed tomography (TACT) is superior in detecting signals that were not appropriately visualized by other available imaging modalities. It offers increased sensitivity and specificity for a number of diagnostic tasks, high resolution, lower dose after image processing, and no artifacts associated with CT.[27]

Ultrasonography: Used in periodontology to measure alveolar crest height and assess periodontal bone morphology. However, it may not be accurate or reproducible, especially in difficult-to-access areas.[28] Palou et al. (1987) were also able to assess periodontal bone morphology using ultrasonic imaging. They concluded that measurements of the alveolar bone topography with ultrasonic probes are not accurate and reproducible, especially in difficult access areas.[29] Lost et al. (1988) were able to determine the width of the periodontal ligament, using dimensional in prepared pig jaws. A reliable image of the alveolar crest of the gingival ligament tissues and the entrance of the periodontal space could be obtained by ultrasound.[30]

Micro CT used to quantify histomorphometry of alveolar bone.[31] It allows for high-resolution imaging of specimens at the micron level and enables computer-aided reorientation following scanning, ensuring almost exact alignment.

Bone Scanning or Radionuclide Imaging: Detects new bone growth or breakdown areas using nuclear scans. It is used to assess damage to the alveolar bones and monitor conditions affecting the periodontium.[32]

SimPlant**:** Computer program for assessing dental implant sites. It uses CT data combined with advanced computer graphics to assess bone volume, height, and quality, select appropriate implant length, and visualize the lower alveolar canal clearly.[33]

Intraoral Scanners: Project an illumination source to the object for a scan and send photomorphological data to a connecting computer system to generate 3D models in digital form.[34,35]Zhang et al. 2021 indicated that gingival volume changes could be measured with an intraocular scanner after therapy. Other parameters such as Probing depth, bleeding index and keratinized gingival width have been positively correlated with the results.[36]The Key benefits include shortens the time needed for diagnosis,

provides relief from pain and discomfort to patients, enables real-time scanning and visualization and allows for quick diagnosis and communication without potential deformation of results.[37] Commercially available intraoral scanners include the TRIOS, the iTero Element, CEREC Omnicam and the Emerald.

2.3. Enhancements in intraoral ultrasound devices[38]

The Krupp SDM, the SonoTouch, the IO3-12 and the UltraSonographic Probe are some examples of advanced ultrasound devices used for intraoral applications. These devices are commonly used in clinical practice to measure lesions in the gingiva, tooth fractures, superficial tissue lesions, maxillofacial and alveolar bone defects, as well as periodontal thickness. Endoscopic capillaroscopy is a technique used to assess periodontal health. It involves inserting a submillimeter-sized optical fiber into the periodontal pocket crevice for in vivo imaging and recording of the microvasculature. This method employs green light with a wavelength of 520 nm, which is absorbed by both oxygenated and deoxygenated blood. Consequently, blood vessels containing red blood cells appear dark against a green background, enabling the acquisition of high-resolution images of periodontal pocket microcirculation.[39]

2.4. Improvements in chairside diagnostic tests

These assessments are performed prior to dental treatment to establish the baseline values of destructive activity in the periodontal pocket, which can be compared with post-treatment values. These kits have been used in long-term studies to detect elevated levels of gingival crevicular fluid (GCF) and saliva markers, which serve as indicators of periodontal disease activity which offers the advantage of predicting disease activity in the periodontal pocket, easy to use, particularly for color detection, provide quick readings after a short time and used to educate patients about the disease condition.

2.4.1. Disadvantages

1. Predicting disease activity at the site can be challenging.
2. Selecting the biomarker to evaluate is challenging because no biomarker has been proven to be an exact indicator of disease activity.

PerioGard and PocketWatch are products designed to measure the levels of aspartate aminotransferase (AST), which is an abundant enzyme found in human gingival epithelial cells and gingival fibroblasts. Elevated AST levels in gingival crevicular fluid after cell death may indicate significant gingival tissue destruction, as large amounts of AST are released from the cells' cytoplasm into the GCF. The activity of AST in both products is evaluated by comparing the color of the collected GCF from patients with that of the controlled AST-positive group, based on the enzymatic catalysis reaction.

The PerioSafe and ImplantSafe tests are qualitative evaluations of aMMP-8 levels in oral rinse and gingival crevicular fluid respectively. These tests use an automated digital device called Oralyzer, which can quantify the amount of aMMP8 in ng/mL units within five minutes. Recent reports indicate that a device equipped with an MMP-8 assay kit is beneficial for distinguishing between active and inactive sites through rapid and straightforward analysis. It is also useful for detecting asymptomatic, ongoing periodontitis before clinical and radiographic signs become apparent.[40–42]

SillHa and Salivary Multi Testan**,** assess a range of saliva indicators (including blood, leukocytes, and proteins) linked to gingival health. Both products comprise test strip kits and an automated wavelength reflectometry device. This device detects color changes on the test strips in about five minutes.

Electronic Taste Chips - These are chemically sensitized bead microreactors integrated into a lab-on-a-chip system. They have the ability to distinguish between healthy individuals and those with periodontal disease based on their CRP level. Furthermore, they can concurrently monitor saliva inflammation biomarkers. The system analyzes analytes such as acids, bases, electrolytes, and proteins in the solution phase.[43]

Oral Fluid NanoSensor Test- In 2012, Dr. David Wong created OFNASET, an automated device designed for the detection of oral cancer using saliva. The Oral Fluid NanoSensor Test (OFNASET) detects various salivary proteins and nucleic acids using an electrochemical method. It is capable of identifying four salivary mRNA biomarkers (IL-8, ODZ, SAT and IL-1b) and two salivary proteomic biomarkers (Thioredoxin and IL-8) present in saliva.

Integrated Microfluidic Platform for Oral Diagnostics (IMPOD) assists in rapidly quantifying salivary biomarkers in small 10 ml volumes within 3 to 10 minutes. This includes the rapid assessment of levels of the collagen-cleaving enzyme MMP-8 in saliva from individuals, both healthy and with periodontal disease.

Periocheck**-** In US, Periocheck has received approval from the Food and Drug Administration. It is the quickest chairside test in GCF for neutral proteases like elastases, proteinases, and collagenases. Elevated levels of these enzymes in gingival crevicular fluid (GCF) are observed during the development of gingivitis and at sites with existing periodontitis.[44]

IAI Pado Test 4.5 used to identify four types of periodontal pathogens: Aa, Pg, Tannerella Forsythia, and T. denticola. This test utilizes oligonucleotide probes that specifically target the 16S rRNA gene, responsible for encoding the ribosomal RNA subunit of bacterial ribosomes, through a process called DNA hybridization.

Omnigene**,** a nucleic acid technology genomic probe that utilizes genetic engineering to create species-specific DNA probe tests for eight periodontal pathogens, using DNA hybridization.

Evalusite (kodak)**,** a membrane-based enzyme immunoassay used to detect three dental pathogens, available on the market.In this assay, the antigen is linked with a membrane-bound antibody to form an immunocomplex, which is then detected using a colorimetric reaction.[44]

PerioScan**,** a diagnostic test kit that employs the BANA (N-benzoyl-DL-arginine-2-naphthylamide) hydrolysis reaction to detect bacterial trypsin-like proteases in dental plaque. It detects the presence of three periodontal pathogens in subgingival plaque (T. denticola, P. gingivalis, and B. forsythus). The BANA test was developed by Dr. Walter Loesche and colleagues at the University of Michigan. Among the 60 bacterial species in the subgingival microbiota, only these three bacteria possess a trypsin-like enzyme that hydrolyzes the synthetic peptide benzoyl-DL-arginine-naphthylamide. The test involves a plastic strip with two separate reagent matrices attached. The lower white reagent matrix is impregnated with N-benzoyl-DL-arginine-B-napthylamide, onto which subgingival plaque samples are applied. Following a 5-minute incubation at 55°C, the upper buff reagent matrix reacts with one of the hydrolytic products of the enzyme reaction, using a chromogenic diazo reagent, resulting in a permanent blue color. The intensity of this blue color in the upper buff matrix determines the strength of the reaction, indicating whether it is positive or weak.[45]

Toxicity Prescreening Assay detects the presence of bacteria indirectly by identifying two markers of gingival infection: bacterial toxins and proteins. The test is associated with the extent of inflammation and the advancement of the destructive process. As the concentration of these toxins increases, a change in the color intensity scale, based on metabolic activity, determines whether the periodontal disease is active or inactive.[46]

2.5. Developments in genetic testing

Some of the genes frequently associated are the Interleukin-1,6 gene, TNF-α gene, Fc receptor gene, N-formyl peptide receptor gene, Vitamin D receptor gene, Human Leukocyte Antigen gene, N-acetyl transferase gene, and Matrix Metallo Proteinase (MMP) gene.[47] The presence or absence of these genes can be detected by subjecting the patient to genetic testing. The Human Genome Project has opened new potential territories to be explored in the identification of diseased genes or disease-causing genes, such as the candidate gene approach.[48]The candidate gene approach is a method used to determine the presence or absence of a known gene.

For this purpose, laboratory techniques such as Polymerase Chain Reaction (PCR), DNA sequencing, and Fluorescence in situ hybridization (FISH) are utilized.

1. Polymerase chain reaction (PCR) involves in vitro cycles of oligonucleotide (primer)-directed DNA synthesis of "target sequences." It is considered the fastest and most sensitive method for detecting bacterial DNA sequences. A variation of PCR called "real-time PCR" enables the detection and quantification of specific microorganisms in plaque.
2. DNA sequencing involves identifying the nucleotide sequence responsible for a specific gene or DNA. Common methods include Sanger sequencing, developed by British biochemist Fred Sanger and colleagues in 1977, and Next-generation sequencing, which comprises a set of newer DNA sequencing technologies.[49]
3. Fluorescence in situ hybridization (FISH)- gene mapping technique that employs fluorescent probes to identify a specific gene. This method can detect both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) sequences, with fluorescence being detected using fluorescent microscopy. Limitations are requirement of equipments and infrastructure, expensive and patient incompliance.[50]

Molecular Biology Techniques - Analysis of DNA, RNA, and protein structure and function is conducted using principles from molecular biology techniques. Diagnostic tests necessitate specific DNA fragments to identify complementary DNA sequences specific to the target organisms. This technology involves amplifying bacterial DNA from the plaque sample using a specific genetic sequence of the target pathogen.[51]

(a) Nucleic acid probes - Single-stranded nucleic acid molecule (DNA or RNA) from a specific pathogen that is synthesized and labeled with an enzyme or a radioisotope. Hybridization refers to the pairing of complementary DNA strands, leading to the formation of double-stranded DNA.

(b) Checkerboard DNA-DNA hybridization technology used to detect and quantify specific 40 bacterial species using whole genomic digoxigenin-labeled DNA probes, associated with periodontal disease. It allows for the simultaneous assessment of multiple species, providing valuable information for diagnosis and treatment planning.

(c) Analysis of saliva or peri-implant fluids specimens include, LAB SRL, GEN- TREND, Oral DNA Labs, Carpegen, OralVital, Perio Prevention.

3. Conclusion

The accuracy of diagnosis is crucial for the effectiveness of any treatment. Presently, the existing diagnostic methods can effectively manage most cases of chronic periodontitis. However, physicians need to ensure that patients will benefit from these tests, considering both diagnostic information and the costs in terms of time and money. Advanced diagnostic techniques will play a crucial role in the future by improving the documentation of disease activity and expanding treatment options. Despite significant advancements in diagnostic methodology, conventional procedures remain the gold standard for evaluating diseases.

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5. Conflict of Interest

None.

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