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Point of Care Microbiological Tests in Periodontology

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Abstract

Periodontal diseases involve a complex inflammatory response of the periodontal tissues against various micro-organisms leading to destruction of the tooth supporting structures. Laboratory investigations have remained the mainstay for analytical processes of a large number of samples involving various disciplines. However, due to the limitations regarding health-care budgets, there has been a paradigm shift from central laboratories to point of care tests (POCT). Newer diagnostic tests can prove to be beneficial in evaluation of patient's response to periodontal therapy. Oral fluid-based POCT used selectively are now being utilized as the potential "chairside" tests for determination of oral diseases.

Keywords: Point of care tests, Periodontology, Microbiology

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Introduction

Periodontal disease comprises of a complex inflammatory response of the periodontal tissues against various micro-organisms which leads to destruction of the supporting tissues around the teeth.[1] Oral health of 10% of the world's population is compromised due to severe periodontitis and its association with systemic health.[2] Clinical diagnostic procedures for periodontal and peri-implant diseases such as measurements of pocket depth, attachment loss, and bleeding on probing and radiographic examination assess past tissue destruction without any information about the current disease status or future progression. This is further complicated by the episodic progression of the disease course.[3] Chair side detection and quantification methods pose a challenge till date due to issues of reliability and validity.[4] An accurate diagnosis is the first definitive step toward the planning and execution of a suitable individualized treatment plan, which contributes significantly toward the success of the therapy.[5] Clinical diagnostic parameters that were introduced more than half a century ago continue to function as the basic model for periodontal diagnosis in the current clinical practice as well.[6] Laboratory testing has remained the mainstay for analytical processes of a large number of samples involving various disciplines. Due to the limitations and pressure on health-care budgets, there has been a paradigm shift from the central laboratories to point of care tests (POCT).[7]

Newer diagnostic tests can be beneficial in evaluation of patient's response to periodontal therapy.[8] Oral fluid-based POCT used seldom earlier are now being utilized as the potential "chairside" test for determination of oral diseases.[9]

POCT can be defined as testing performed close to the patient at the time when care is required.[10]

Among the various microbes causing periodontal infections, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* are implicated in causing systemic infections such as cardiovascular diseases, rheumatoid arthritis, and severe pregnancy outcomes. The development of microfluidic approaches and detection of biomarker molecules in the oral cavity has made oral-based POCT methods for diagnosis, a necessity.[11,12]

Microbiological POCT use advanced techniques such as PCR for ribonucleic acid (RNA) and DNA and ELISAs for proteins in the saliva or gingival crevicular fluid and can be of the following types.[9]

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My Perio Path

My Perio Path 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.[13]

Omnigene

OmniGene Diagnostics, Inc. (DNA Agenotek™. Ottawa, An, Canada) are species specific DNA probes and can identify eight pathogens causing periodontal disease.[14]

1. *P. gingivalis*
2. *Prevotella intermedia*
3. *Aggregatibacter actinomycetemcomitans*
4. *F. nucleatum*
5. *Eikenella corrodens*
6. *Campylobacter rectus*
7. *Bacteroides forsythus*
8. *Treponema denticola*

Subgingival plaque is obtained on a paper and placed in the container and assayed.

Perioscan (BANA)

(N-benzoyl-DL-arginine-2-naphthylamide) periodontal test (Ora Tec Corporation Manassas, USA).

This test detects bacterial trypsin-like proteases in the dental plaque produced by *P. gingivalis*, *T. denticola, Tannerella forsythia,* and some *Capnocytophaga* strains.[15] It can be measured with the hydrolysis of the colorless substrate N-benzoyl-DL-arginine-2-naphthylamide. When the hydrolysis takes place, it releases the chromophore β-naphthylamide which turns orange red when a drop of fast garnet is added. The need for periodontal therapy to reduce the risk of adverse pregnancy outcomes can be addressed using this test in routine ante-natal check-up. In a study by Turton *et al*. in 2017, significant differences in pregnancy outcomes of BANA negative and BANA positive mothers were reported.[16]

Evalusite (Eastman Kodak Company, Rochester, NY, USA)

Three putative periodontopathogens (Aa, Pg, and Pi) can be detected using membrane-based enzyme immunoassay, Evalusite. The kit detects the presence of the pathogens based on an antibody bound sandwich type enzyme linked immunosorbent within 10 min.[17]

Perio 2000

Degradation of serum proteins (cysteine and methionine) leads to volatile sulfide compounds (VSCs) production by microorganisms such as *P. gingivalis*, *P. intermedia,* and *T. forsythia*. Evaluations of VSCs are indicative of subgingival microbial load as it plays role in degrading periodontal structures aggravating periodontitis. Perio 2000 system displays the sulfide level digitally at each site. Sterile wash solution is used to hydrate the tip and it is inserted sub gingivally. After obtaining the reading, the tip is washed and reinserted in another subgingival site.[18]

Toxicity Prescreening Assay (TOPAS)

TOPAS is a chairside test kit for indirectly detecting bacterial toxins and bacterial proteins which are one of the markers for the presence of gingival infection. The principle behind this test relies on the detection of actively dividing and growing pathogens which can be assessed through the metabolic activity of these organisms in the crevicular fluid. This test can be used to know difference between an active and an inactive periodontal disease as indicated by the change in the color intensity scale of the test based on the fact that metabolic activity increases as the concentrations of these toxins increases.[19] It detects the indirect presence of bacteria by two markers of gingival infection which are bacterial toxins and bacterial proteins.

MMP Dipstick Method

This test helps in detection of MMPs in GCF. MMPs are host-derived proteinases which plays a major role in periodontitis and dental peri-implant health and diseases. This forms the basis for the development of both qualitative and quantitative chairside POC technologies which 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 POC immunotests for oral fluid and serum MMP-8 analysis. The MMP-8 stick-test can differentiate healthy gingiva and gingivitis sites from periodontitis sites and the results obtained correlates with that of quantitative laboratory Immunofluorometric Assay.[20]

MMP-8 dipstick is based on the immunochromatography principle that uses two monoclonal antibodies specific for different epitopes of MMp-8. The test stick results can be detected in 5 min. The antibody detects both neutrophils and non-PMN type MMP-8 isoforms. The GCF sample collected is placed in a test tube containing 0.5 ml of a buffer at pH 7.4. When the dipstick is placed in the extracted sample, the dipstick absorbs liquid, which starts to flow up the dipstick. When the sample contains MMP-8, it binds to antibody attached to the latex particles. The particles are carried by the liquid flow if MMP-8 is bound to them, they bind to the catching antibody. If the concentration of MMP-8 in the sample exceeds the cutoff value for the test, a positive line will appear in the result area.

Oral Fluid Nano Sensor Test (OFNASET)

OFNASET or lab-on-a-chip nanotechnology is a microelectromechanical system that is capable of detecting salivary protein and RNA biomarkers. Microfabrication technology has led to the development of electrochemical biosensors with the capacity for sensitive and marker-specific detection of nucleic acids and proteins. Application of universal molecular analysis for cancer screening helps in the early recognition of cancer, which can significantly reduce the mortality and morbidity associated with cancer. The intended use of the OFNASET is for the detection of salivary biomarkers for oral cancer — two salivary proteomic biomarkers (thioredoxin and interleukin-8 [IL-8]) and four salivary mRNA biomarkers (SAT, ODZ, IL-8, and IL-1b). This electrochemical sensor contains capture and detector probes to target or to bind with antibodies related to cancer antigens. The capture probe anchors the target to the sensor, whereas the detector probe signals the presence of the target through a reporter molecule.[21-23]

Electronic Taste Chips

This microchip-based detection system is used for measuring analytes (acids, bases, electrolytes, and proteins) in solution phase. On the interior regions of the microspheres, sensor array platform is placed where all the chemical and immunological reactions are performed. These microspheres are located on the inverted pyramidal microchambers of microchip. A charge-coupled device video chip visualizes and captures the various optical signals generated by the reactions on the microspheres. The ETC system has the advantage over the ELISA in having porous beads, which allows greater number of antibody molecules to capture and thus detect, CRP at extremely low concentrations.[9,24]

Integrated Microfluidic Platform for Oral Diagnostics

A clinical point-of-care diagnostic test that involves a monolithic disposable cartridge designed to perform in a compact analytical equipment to identify an oral disease biomarker in human saliva. To evaluate analyte concentrations in pre-treated saliva samples, it incorporates sample pre-treatment (filtering, enrichment, and mixing) alongside electrophoretic immunoassays. Photoinitiated polymerization is employed to coat the channel surfaces with the help of linear polyacrylamide that undergoes cross-linking *in situ*. It rapidly measures MMP-8, IL-6, and TNF-α in saliva from healthy and periodontally diseased subjects.[25,26]

Salivary Diagnostic and Research Assay Kits (Salimetrics)

It helps in the estimation of cytokines including IL, MMPs, and so forth and various hormones including cortisol, cortinine, DHEA, testosterone, estradiol, progesterone, and estriol in saliva.[27]

Perio Safe

The lateral flow chromatography aMMP-8 oral fluid PoC–immunotests, identifies and screens chronic and initial periodontitis sites and patients, differentiates active sites and patients, predicts disease progression, and can be utilized to monitoring the treatment and medication as well assuring maintenance. In addition, it identifies genetically predisposed adolescents.[28]

Implant Safe

The Implant Safe a MMP-8 (also a rapid lateral flow chromatography immunotests), is a modern, *in vitro* diagnostic dip-stick test for use in dental implantology. This PoC test is a rapid test for routine implant checkups as a part of a regular implant maintenance program. The test can provide valuable information for preventive care through early detection of a risk of hidden inflammation with consequent tissue and alveolar bone break-down. Early detection provides the opportunity for timely treatment to arrest the development of mucositis or peri-implantitis.[28]

Genetic Test

Periodontitis susceptibility trait test

The periodontitis susceptibility trait test is the first genetic susceptibility test for severe periodontitis. It is commercially available. It evaluates the simultaneous occurrence of allele 2 at the IL-1α+4845 and 1β+3954 loci. 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.[29]

MyPerioID

MyPerioID identifies the genetic susceptibility of the patient to periodontal diseases using salivary samples which are shipped to the laboratory for the results. These test plays a role in evaluating the patients which are at higher risk of periodontal destruction.[13]

MyPerioID test uses saliva to determine a patient's genetic susceptibility to periodontal diseases. It assesses patients which are at higher risk of more serious periodontal infections. This test requires the transportation of saliva samples to a laboratory for results.[30]

a. Detects (from human DNA) genetic variation/polymorphism within the IL-1 gene
b. IL-1 is a major inflammatory mediator
c. 30–35% of the US population has this genetic variation
d. IL-1 positive individuals tend to have more aggressive and more severe infections
e. Determines patients that are most susceptible to severe disease, especially if the patients' smoke
f. This genetic variation can increase risk for severe disease or tooth loss by 2–7 times when present.

PCR on a Chip

Gaertig *et al.* have developed a model point of care device based on the polymerase chain reaction of DNA isolated from periodontal pathogens and examined to precisely detect species-specific sequences on a rotating chip with lyophilized reagents for polymerase chain reaction. The detection and quantification of periodontal bacteria amplification were carried out using a Light cycler 480 II. The PCR was implemented on 1 mm thick PC chips with cavities that hold a volume of 10 µl of reaction mix. The chips were sealed with sealing foils (nerbe plus GmbH, Winsen/Luhe, Germany) from both sides with the PCR mix embedded.

For amplification, the PCR chip rotates on the thermocycling Device. Briefly, it is composed of six circularly arranged pie-slice-shaped heating blocks, three of them for accomplishing denaturation, annealing, and elongation steps and three additional blocks for achieving rapid temperature changes inside the cavities. The simple and model point-of-care system can be easily applied in every dental office, reduces both time and costs for diagnostics, and eliminates the necessity for transport to external laboratories and therefore facilitates crucial fast treatments in the future.[31]

Conclusion

Periodontal point of care diagnostic kits offer rapid, reproducible mode of diagnosis, and the results can be used for patient motivation as well. They are useful especially in monitoring patients post-treatment, for evaluating the response to therapy and disease recurrence. These devices will also enable masses to be screened, particularly underserved communities and resource limited areas. Such applications might serve better for identification of at-risk groups and increase access to treatment for those most in need, improving public health in periodontology and the oral health field in general. A careful analysis is mandatory, before adopting any newly emerged diagnostic test in the current clinical protocol. The novel test must be weighed against the conventional criteria of diagnosis in its sensitivity, specificity, validity, and reliability. The benefit of having a particular piece of diagnostic information must not only outweigh the effort to obtain it on the level of each individual; however, the impact of a new diagnostic procedure should also be evaluated at a more global level, to maximize the overall benefit of the total investment in healthcare. Adequate guidelines for the use of diagnostic routines should be issued and implemented from regulatory bodies in health care. Nevertheless, as new procedures are introduced in periodontology during these times of cost containment in healthcare, practitioners must use caution in deciding which particular patients would benefit from a comprehensive evaluation. Since the emphasis is switching more toward prevention and early detection of a variety of diseases, development of these devices has made a dramatic impact on health-care services. The next decade will bring breakthroughs in terms of precision, efficiency, and bedside monitoring instead of hospital setups.

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