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SAVREMENA DOSTIGNUĆA U MIKROBIOLOŠKOJ DIJAGNOSTICI TESTOVIMA PRIMENLJIVIM U STOMATOLOŠKOJ STOLICI

RECENT ADVANCES IN MICROBIOLOGICAL DIAGNOSTIC CHAIRSIDE TESTS: AN INFORMATIVE REVIEW

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Sažetak

Uvod: *Napredak u dijagnostičkim procedurama za periodontalne bolesti od suštinskog je značaja za tačno prepoznavanje, praćenje i efikasno planiranje terapije. Razvoj novih dijagnostičkih setova mogao bi unaprediti mogućnost identifikacije aktivnih oboljenja, predviđanja buduće progresije bolesti i procene odgovora na periodontalnu terapiju, čime bi se omogućilo personalizovano i efikasnije lečenje.*
Cilj *ovog rada bio je da se kliničarima pruži pregled koristi koje setovi za dijagnostiku na stolici (chairside kits) donose u svakodnevnoj kliničkoj praksi.*
Materijal i metode: *Informacije korišćene u ovom radu prikupljene su iz relevantnih izvora znanja, internet baza podataka, pretraživača i alata, te analizirane kao osnova za dokumentovano sagledavanje dostupnih dokaza i mogućnosti primene dijagnostičkih setova u parodontologiji.*
Rezultati: *Svaka pretraga treba da započne jasno formulisanim istraživačkim pitanjem. U cilju pronalaženja pouzdanih odgovora, predstavljeni su potencijalni izvori zasnovani na dokazima, poput PubMed/MEDLINE baze podataka, Cochrane Library i Google Scholar pretraživača. Rezultati su pokazali da su setovi za dijagnostiku na stolici efikasni u ranoj detekciji subgingivalne mikrobiote iz uzoraka dentalnog plaka.*
Zaključak: *Ova pregledna analiza obuhvata različite mikrobiološke dijagnostičke setove za primenu na stolici, uključujući i point-of-care sisteme, koji mogu značajno doprineti preciznijoj dijagnostici i planiranju terapije u periodontalnoj praksi.*

Ključne reči: *dijagnoza parodontopatije, mikrobiološki testovi, dijagnostički setovi na stolici, point-of-care testovi*

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Abstract

Introduction: *Advancements in diagnostic procedures for periodontal diseases are crucial for accurate detection, monitoring, and effective treatment planning. Developing novel diagnostic kits could enhance our ability to identify active disorders, predict future disease progression, and evaluate responses to periodontal therapy, leading to more personalized and efficient treatment strategies.*
Aim: *The aim was to provide for clinicians an overview of the benefits of chairside diagnostic kits in day-to-day clinical practice.*
Materials and Methods: *Information sources have been gathered, and discussed as a foundation for a documented vision on knowledge questions, online information sources, search engines, databases, and tools.*
Results: *Every search should start with a carefully phrased question. To help find a reliable answer, potential evidence-based online sources were presented, such as PubMed/MEDLINE, Cochrane Library and Google (Scholar). The results suggested that the chairside diagnostic kits were efficient in the early detection of subgingival microbiota from plaque samples.*
Conclusion: *This comprehensive compilation includes various chairside microbiological diagnostic kits, including point-of-care systems, which could be beneficial for periodontal diagnosis and treatment planning.*

Key words: *periodontal diagnosis, microbiological tests, chairside kits, point of care tests*

Introduction

Indeed, the interaction between pathogenic microbes and the host immune response plays a crucial role in the development of periodontal diseases. Researchers have delved into a holistic understanding of how the host's immune system responds to pathogenic bacteria in the oral cavity. Subsequently, this results in an inflammatory-mediated polymicrobial emergence and dysbiotic exacerbation model[1]. This dual focus on microbial etiology and host response enhances our comprehension of the mechanisms underlying periodontitis.

These revelations prompted a demand for diagnostic methods capable of accurately assessing disease activity in patients, marking a significant evolution in periodontal research and clinical approaches. Certainly, the accuracy of periodontal diagnosis is pivotal, influencing the success of subsequent therapy. To enhance this process and predict disease progression, incorporating newer diagnostic tests, including microbiologic, immunologic, systemic, and genetic factors, alongside traditional clinical and radiographic parameters, is crucial. The development of assays for identifying and assessing periodontal pathogens in patient samples reflects ongoing efforts to refine diagnostic approaches and tailor treatments effectively.

The focus on microbiological chairside kits in this consolidated review is well-founded. These kits offer clinicians valuable and timely information for early disease identification, enabling the formulation of precise treatment plans. This structured approach not only facilitates monitoring during periodontal therapy but also proves beneficial in the maintenance recall phase, enhancing overall patient care and outcomes.

Diagnostic Tests

For a periodontist, a diagnostic test determines whether the disease identified by clinical parameters is active or inactive. There are a few basic terminologies which need to be well defined for a detailed understanding of the topic. The ratio of sites with active disease that are acknowledged as positive by the diagnostic test is known as ‘sensitivity’. The ratio of sites without active disease that are rightly recognized by the diagnostic test is known as ‘specificity’. ‘Predictive value’ is the percentage of time a positive test correctly predicts disease. ‘Negative predictive value’ shows how often a negative test predicts health. There are basically four factual outcomes that can be achieved on comparison of test results of patients diagnosed with or without periodontitis[2,3].

Table 1 shows the variable outcome attained in the presence or absence of periodontal disease.

Chairside Diagnostic Tests

A few of the fundamental characteristics of chairside diagnostic kits have been illustrated in Figure 1.

Periodontal diagnostic procedures provide valuable details to the clinician regarding the nature of the existing periodontal disease, the involved site, and the severity of the disease. Bleeding on probing, probing pocket depth (PPD), clinical attachment loss (CAL), oral hygiene index, and radiographs are traditional landmark clinical measurements used for periodontal diagnosis, which are rather inadequate dimensions since they indicate former periodontal disease status instead of current disease activity. Various diagnostic tests are available for the detection of periodontal pathogens; amongst these, chairside tests offer rapid results compared to traditional laboratory processes[4].

Table 1. Variable outcome attained in presence or absence of periodontal disease

Outcome	Diagnosed with periodontal disease	Absence of periodontal disease
Characteristics of disease present	True positive	False negative
Characteristics of disease absent	False negative	True negative

Figure 1. Chairside diagnoshcs kits

1. ***Microbiological Test Kits***

The diagnosis of various forms of periodontal disease is supported by the microbiological tests that are directed towards the elimination of periodontopathogens. These provide guidelines for disease origination and help in the determination of periodontal sites that are at greater risk for active destruction.

The following are a few of the chairside test kits:

Omnigene[5]

The early 1980s marked an era focussed on microbial etiology as the basis of periodontal disease, and a demand for newer therapeutic methods to supplement conventional periodontal treatment. During this course, market analyst, Dr. Lynn Klotz at BioTechnica International, Inc. (BTl) identified a tool to provide enhanced microbiological information to dental clinicians, which could be used as a guide for diagnosis and treatment strategies for periodontal disease. It has been well documented that the predominant pathogen for periodontitis belongs to the anaerobic species, and its procurement from clinically diseased sites is difficult to cultivate or culture. Hence, a technical assay was explored which did not require viability of the species for the identification and quantification of the clinical samples. Since the understanding of periodontal disease confirmed its slow progressive process with the absence of mortality, an off-site assay kit was tested using recombinant DNA technology, which worked on the principles of genetic engineering. This information is directed towards the alternative of the DNA probe technique as the foundation for the test.

The DNA probe systems identify several known periodontopathogens. However, the development of species-specific DNA probe tests was labelled for eight periodontal pathogens, namely Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Aggregatibacter actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn), Eikenella corrodens (Ec), Campylobacter rectus (Cr), Bacteroides forsythus (Bf) and Treponema denticola (Td).

The advantages stated are fewer efforts on the part of the clinician, wherein subgingival plaque samples can be collected and sent to OmniGene Diagnostics, a licenced clinical laboratory. Once the data is analysed, the information can be quickly transferred to the clinician via emails, phone or fax. Thereafter, it reduces the time required for commencement of appropriate treatment.

Al Yahfoufi Z and Hadchiti W assessed the prevalence and association of three putative periodontal pathogens, Aa, Pg and Pi, in a group of subjects diagnosed with minimal periodontal disease with no history of periodontal treatment. The methodology involved the procurement of subgingival plaque samples on a sterile paper point from the deepest pocket. Further, DNA probes (Omnigene) was utilized for specific identification and quantification of Aa, Pg, and Pi. The results showed 23% of samples presented with positive Aa comitans; 79% of plaque samples had Pg, and 82% of plaque

samples contained Pi. A significant association was observed between the presence of Aa comitans and Pg ($p = 0.016$). The authors concluded that a high frequency of the three periodontal pathogens (Aa, Pg, and Pi) was observed in the plaque samples[6].

Micro Probe Corporation

This company has created an in-office nucleic acid probe assay to semiquantitatively detect periodontal pathogens. Patient plaque samples are treated to lyse bacterial cells through heating with detergent. The extracted DNA is loaded into the initial well of a multiwell cassette, which is then inserted into a machine featuring a programmable robotic arm. The device provides a digital readout of the current bacterial load[7].

Evalusite

A chairside kit employs membrane-bound immunoassay for the detection of three popular and prevalent periodontal pathogens, namely, Aa, Pg and Pi. The identification of bacterial organization in subgingival plaque requires a distinct plan for microbiological sampling due to the large number of potential sample sites within periodontal patients. Approximately 5 minutes are needed to identify and separate the bacteria, following which the results can be visually translated. The plaque sample procured from the patients is prepared by the addition of a detergent, and then the mixture is spread through a filter into a reagent well of the assay test kit. Membrane-bound antibody in the well specific to Aa, Pg, and Pi reacts with the plaque sample. Antigen and antibody complexes formed on the membrane are detected by the addition of an enzyme-labelled second antibody together with a coloured enzyme substrate. Separate dots indicate the presence of 3 different species[8].

Perioscan

Another diagnostic test kit which provides detection of bacterial trypsin-like proteases in the dental plaque by using the BANA hydrolysis reaction. One of the contemporary replacements to bacterial cultures is the microbial-enzymatic BANA (N-benzoyl-DL-arginine-2-naphthylamide) test. This test is extremely susceptible, noticing lesser amounts of pathogens. However, within the subgingival biofilm, it recognizes the presence of three periodontal pathogens, which include Td, Pg and Tf. The peptide analog BANA can be hydrolysed by the peptidases of these three bacterial species. The collection of subgingival plaque specimens should be carried out cautiously using either a gracey curette or a paper point. The specimen should be procured from at least 4 teeth with maximum probing depth. A sterile piece of cotton or any other suitable cleanser is used to wipe the curette to prevent carry-over of plaque before taking another specimen. The contents of the test kit include reagent strips that are plastic cards. Separate reagent-containing matrices are affixed on the plastic cards. B-naphthylamide is implanted in the upper strip of the test and is one of the hydrolytic outcomes of the effect, which reacts with a reagent and creates a permanent blue colour. Two separate reagent matrices are attached to a plastic strip. The lower white reagent matrix is soaked with BANA onto which the subgingival plaque samples are smeared. Then saline solution is used to moisten the upper matrix, and the test is pleated so that the two matrices are impending in contact. It is then incubated for 5 minutes at 55 °Celsius. A chromogenic diazo reagent is present on the upper buff reagent matrix and reacts with one of the hydrolytic outcomes of the enzyme reaction, forming a blue colour. The concentration of bacterial species also propitiates the color[4]. The permanent blue colour appears in the upper buff matrix. The intensity of the colour indicates if the test is positive, negative or a weak reaction.

In 2018, Fenol A et al.[9] conducted a trial to compare and detect the presence of periodontal pathogens in chronic periodontitis patients after nonsurgical periodontal therapy with and without the use of diode laser disinfection using the BANA test. Subgingival plaque specimens were applied onto the test strip using a curette. The change from colourless to blue indicated the presence of periodontal pathogens. The authors observed a reduction of the key pathogens in both groups at the end of 2 weeks. Further, at the end of 2 months, the test group showed a more statistically significant reduction. Hence, the conclusion derived was that BANA-enzymatic kit was a simple chairside kit that could be reliable indicator of BANA positive species in dental plaque.

In another study in 2017, Turton MS et al.[10] tested the hypothesis if BANA diagnostic test for periodontal disease could be used as an indicator of the risk of adverse pregnancy outcomes in mothers attending antenatal clinics. Plaque was collected and wiped onto the BANA impregnated strip.

Significant differences were found between the pregnancy outcomes of BANA-negative and BANA-positive mothers. The sensitivity and negative predictive values were 87% and 91%, respectively. In detecting low birth weight, the sensitivity ranged from 75% to 78%. For identifying preterm delivery and preterm low birth weight delivery, the sensitivity and negative predictive values were 87% and 94%, respectively.The authors concluded that the BANA test signifies the need for periodontal therapy so as to reduce the risk of adverse pregnancy outcomes and could form part of the routine antenatal examination.

My Periopath

MyPerioPath is a point-of-care (POC) device manufactured by Oral DNA Labs for detecting periodontal disease-causing pathogens in saliva samples. This test utilizes the DNA polymerase chain reaction to identify the species and concentration of bacteria in the saliva samples[11].

Iai Pado Test Kit 4.5

The IAI Pado RNA probe test kit (Institut für Angewandte Immunologie, Zuchwil, Switzerland) is useful for the identification of the four widespread periodontal pathogens. This chairside assay test uses an oligonucleotide probe corresponding to sustained remnants of the 16S rRNA gene that encrypts the rRNA, which belongs to the subcategory of bacterial ribosomes. These tests have detection limits which are 103 for Aa and 104 for Pg, Tf and Td. Associated with the checkerboard technique, the identification frequencies attained with this assessment showed a minimal sensitivity of the Pado Test 4.5 technique[12].

In 2017, Pretzl B et al. evaluated the intra-test agreement of pooled samples from the deepest periodontal pocket of each quadrant with a commercially available test kit based on hybridization of 16S rRNA. Subgingival plaque samples collected on two sterile paper points were placed into two separate vials, which were immediately sent for detection by using IAI Pado-Test 4.5. Cohen's κ for detection and counts Tf and Td presented a perfect agreement. Pg showed a substantial agreement, whereas Aa demonstrated a good agreement. Test results of the commercial 16S rRNA test were perfectly reproducible regarding the detection of periodontopathogens[13].

Recent Advancements in Micro-biological Diagnosis

Point-of-Care Test (POCT)

Currently, in periodontal diagnosis, Point of Care Test platforms can be allotted into three groups: Lab-on-Chip (LOC), paper-based platforms, and chairside tests. POCT in periodontitis can help in early detection of the disease, monitoring disease progression, and evaluating the effectiveness of treatment. It can also improve patient outcomes by facilitating timely intervention and personalized treatment planning.

Lab-on-Chip (LOC) Platforms

Chairside Lab-on-a-chip (LOC) technology is poised to play a significant role in detecting biomarkers in saliva, which could enhance global periodontal health efforts. A technique for operating smaller quantities of solutions by contracting and assimilating compound lab actions into a tiny microchip is known as Microfluidic LOC[14]. Currently, immunoassays are the chief method used in the LOC program for diagnosing periodontal disease. Microfluidic LOC techniques have been advanced swiftly and employed extensively in several domains due to their incorporated elements, minute specimens, reagent volume, and quick response. A LOC platform was developed for measuring three salivary biomarkers (MMP-8, IL-1b, and C-reactive protein) to diagnose periodontal disease, utilizing a combination of a microfluidic chip and a fluorescence-based visual method[15].

Impedimetric Antimicrobial Peptide-Based Sensor

This biosensor detects the presence of bacteria using specific peptides. It combines miniaturized and integrated impedimetric transducers with antimicrobial peptides (AMPs) to monitor bacterial colonization with high sensitivity. The biosensor operates within a frequency range of 100 Hz to 1000 kHz with a 100 mV voltage excitation, using the Quadtech 7600 Plus LCR Meter. It is capable of detecting bacteria at concentrations of 101 colony-forming units (CFU) per milliliter in KCl solution and 102 CFU per milliliter in artificial saliva.

The AMPs, when coupled with 3D-IDEA biosensors, demonstrate rapid implementation, label-free detection, and

sensitivity in detecting the periodontitis-associated pathogenic strain S. sanguinis. The AMP-based sensor array can detect a wide spectrum of bacteria and serves as a tool to prevent the initial formation of biofilm, thereby reducing the risk of implant-related infections commonly found in periodontitis patients[16].

Paper-Based Platforms

Paper-based platform is one of the most cost-effective technology with the simple fabrication and is independent from external instruments.

Magnetic-Nanobead-Based Assay

A magnetic-nanobead-based assay, labeled with a gingipain-specific peptide, is utilized for diagnosing infections related to periodontitis caused by P. gingivalis, a common pathogenic bacterium. Gingipains are specific proteases released by the bacteria during inflammatory stages. The sensor is linked with gingipain biomarkers, which are immobilized on a gold biosensing platform through gold-thiol linkage, causing the gold layer's color to change to black. When the immobilized substrates are cleaved by gingipain, the magnet is attracted to the magnetic-nanobeads-peptide fragments, resulting in an observable golden surface color change. This biosensor offers rapid action and higher sensitivity and specificity[17].

Biotechnological advancements have spurred the development of lab-on-a-chip technology and biosensors for analyzing oral biomarkers. These innovations in oral biomarker discovery and validation aim to enhance precision oral medicine by improving diagnosis, prognosis, and patient stratification. Their application has the potential to enhance clinical outcomes for periodontitis and related chronic conditions, benefiting both dental and overall public health[18].

Conclusion

Numerous oral diseases involving carious lesions, endodontic lesions, several types of periodontal diseases, halitosis, and odontogenic infections have well-characterized infectious causes. In the selection of antimicrobial therapy and performing post-treatment periodontal risk assessments for patients with chronic periodontitis, microbiological testing may be predominantly supportive. The accomplishment of every therapy is reliant on the precision of the early analysis. From clinical investigation to further precise, innovative diagnostic procedures, there is a constant evolution in periodontal disease diagnosis. For early diagnosis and treatment, the accessibility of chairside diagnostic test kits will be beneficial. It likewise aids in enhancing patients' amenability for periodontal therapy concerns. Point-of-care testing in periodontitis involves using diagnostic tools or tests to assess the condition directly at the point of patient care, such as in a dental office. These tests are designed to provide quick and accurate information to aid in the diagnosis and management of periodontal disease.

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Abbreviations

Aa—Aggregatibacter actinomycetem-comitans, Pg—Porphyromonas gingivalis, Pi—Prevotella intermedia, Tf—Tannerella forsythus, Td—Treponema denticola, Fn—Fusobacterium nucleatum, Ec—Eikenella corrodens, Fn—Fusobacterium nucleatum, Cr—Campylobacter rectus, Bf—Bacteroides forsythus