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Review Article

Point of Care- Platform For Screening and Detection Using Saliva

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Abstract:

Saliva acts as a mirror of oral systemic health. Hence could be used to monitor the health onset of specific diseases. It is also essential for maintaining the integrity of oral tissue.Saliva contains biomarkers specific for determining state of periodontal health and disease. Any changes in this biomarker could have diagnostic value. Salivary biomarkers detectors can be used for point of care disease screening and detection. This article discusses about point of care technologies specifically developed for salivary diagnostics and describes its current application.

Keywords: point of care platform- salivary biomarker- single biomarker-salivary diagnostic.

Introduction

Periodontitis is a chronic inflammation of the periodontium resulting in breakdown of underneath tissues of the teeth, characterised by loss of attachment and resorption of alveolar bone. As the ability to reconstruct theperiodontium is limited after alveolar bone loss, early diagnosis and intervention should be the goal of the periodontal treatment. However, periodontitis often progresses without noticeable symptoms. Saliva is an optimal biological fluid to serve as a near patient diagnostic tool for periodontitis. Diagnostic test for periodontitis using saliva is now technically feasible with the help of recent development in point of care test. For this test, based on the pathogenesis of periodontitis a panel of optimal biomarkers must be carefully selected. The concentration of biomarkers can be affected by salivary flow rate, circadian rhythm,age, physiological status of the patient.Using saliva for diagnosis of periodontitis has limitation in detecting disease activity at each individual tooth site.In order to overcome this traditional clinical measurement, diagnosis of periodontitis using saliva must be realized as a Point-Of-Care (POC) testing. Feasibilityand development in Point of Care testing are reviewed here.

Periodontitis is a chronic inflammation of periodontium caused by persistent bacterial infection that leads to the breakdown of connective tissue and bone(Ji et al., 2014)[1] As it is chronic in nature, signs and symptoms occur late and the patient often seeks professional careonly after the periodontium is considerably destroyed. Thus, there is a need to diagnose periodontitis in its initial stages using an easy,safe, and readily accessible method. Periodontitis is usually diagnosed using radiograph and clinical methods of probing pocket depth, bleeding on probing and measuring clinical attachment level. Saliva serves as a diagnostic tool for periodontitis.[2] Salivary collection is safe, simple, non-invasive and can be collected repeatedly with minimum discomfort to the patient. Biomarkers identified in saliva correlateswith the clinical parameters of periodontitis. The diagnosis of periodontitis using saliva has a limitation in detecting disease activity at each individual tooth site; traditional clinical measurement is required in order to accomplish this. In this respect, the diagnosis of periodontitis using saliva must be realized as a Point-Of-Care(POC) testing.

Definition:

Point of Care is defined as medical testing conducted outside of laboratory at or near the site of patient care, including the patient'sbedside, the doctor'soffice, and the patients home(Song et al, 2014)[3]

Point of Care would also assist medical doctors in assessing the periodontal status of their patients because periodontitis is associated with many systemic diseases, such as atherosclerosis, coronary heart diseases, diabetes mellitus and rheumatoid arthritis.

Point of Care technologies for molecular diagnostics:

Technologies for detecting biomarker signals in biofluids plays a significant role in advanced diagnostic aids. Technology for molecular diagnosis includes microfluids based device and lab on chip diagnosis. The combination of microfluidic and lab-on-a-chip technologies helps in real time monitoring for biomarkers in a small volume of body fluids. Lab on chip approaches integrate processing steps into one small device. The processing steps in lab on chip includes sampling, sample preparation, detection and data analysis. Microfluidics based devices can analyse samples like blood, saliva, nasal aspirate and urine.

Polymerase Chain Reaction based Point of Care devices are commercially available. For example -- for the detection of pathogens such as influenza, Respiratory Syncytial Virus, Human Immunodeficiency Virus, Methicillin – resistant staphylococcus aureus, Clostridium difficile, and malaria.Nucleic acids, proteins, metabolites and other small molecules of specific pathogens can be detected by Point of Care technologies. By using Polymerase Chain Reaction based Point of Care – nucleic acids can be amplified on chip Polymerase Chain Reaction(non- isothermal) or on- chip isothermalapplication (liy 2006)[4] techniques. Point of Care DNA tests helps to detect genetic mutations associated with various cancers. Microfluidic based technique helps in detection of protein biomarkers which generally relies on antibody based immunoassays. Alternative to antibodies, DNA or RNA oligonucleotides designed to bind to various biomolecules with high specificity and sensitivity. Lateral flow assay is rapid and specific but lack in sensitive and quantitative measures. To improve the sensitivity and specificity new techniques have been developed.Regarding metabolite glucose is the best-known metabolites targeted by Point Of Care testing.

The i- STAT Point of Caredevice quantifies wider range of analytes and sold in millions annually. By using Point of Care technique blood gas, electrolyte and haematology parameters can be electrochemically measured.In 2010, a microfluidic device that measures that nitric oxide has also been developed.Various approaches have been developed to detect the target molecules, most commonly adopted approaches are optical detection and electrochemical detection. Amperometry, potentiometric, and impedimetric measurements willcome underelectrochemical detection. Absorbance calorimetry, chemiluminescence, fluorescence, surface - enhanced Raman scattering spectroscopy and surface plasmon resonance are different optical methods implemented in Point of Care devices.

Biomarkers in saliva must be able to diagnose the presence of periodontaldisease, reflect the severity, predict the prognosis and monitor the prognosis and these are the four basic requirements for a biomarker. And salivary biomarkers can originate from both bacteria and the host. As periodontitis progresses , gingival inflammation , soft tissue destruction and bone destruction occur sequentially and release associated protein or metabolites into the saliva .So the host derived biomarkers are categorized according their reflection of inflammation , soft tissue destruction or bone destruction .The biomarkers that satisfy three of the four requirements are classified as strong (S) .When there is no difference or contradictory results is equal or greater than those with supporting results it is classified as questionable (Q). The remaining biomarkers are classified as potential (P).

Bacteria derived biomarkers:

It includes DNA and proteins. Bytargeting specific area of the 16S rRNA gene levels of well-known pathogenic bacteria like Aggregatibacteractinomycetemcomitans, the three red complex species and several species of orange complex in saliva were determined. Porphyromonas gingivalis, Prevotella intermedia,Tannerella forsythia have been proved as strong biomarker of periodontitis. The presence of P.gingivalis and the activity of dipeptidyl peptidase IV which is a serine protease in saliva has been shown to be associated with periodontitis that cleaves X- Pro dipeptide from the N- terminus of polypeptide chain. DPP4 in saliva is not only bacterial derived biomarker but also host derived including P.gingivalis.

Host-derived Inflammatory Biomarkers

The biomarkers that correlate with clinical symptoms are IL- 1β , MIP- 1α and arginase.[5]Inflammation associated biomarkers are nitric oxide, 8-hydroxydeoxyguanosine, platelet activating factor and fatty acid metabolites, diverse enzymes(arginase ,dipeptidylpeptidaseIV, bglucuronidase,andmyeloperoxidase), antimicrobialproteins(lactoferrinandcalprotectin),inflammatorycytokines(IL-1b, IL-6,IL-18,IFN-g andMIP-1a) andChemerin,C-Reactive Protein,Toll Like Recpectors4, SolubleCD14, and procalcitonin are the proteins thatmediate inflammation.

Host-derived biomarkers associated with soft tissue destruction:

As periodontitis progresses, soft tissues destructionoccurs and several enzymes and proteins that are involved in tissue destruction are released.Among the proteins that are involved in destruction includes MMP-8, MMP-9,HGF, lactate dehydrogenase, aspartate aminotransferase and TIMP -2. They are also a Potential or Strong biomarkers.

Biomarkers associated with soft tissue destruction includes proteins, polysaccharides, lysolipids, fatty acids, monoacylglycerol and uridine(DNA/RNA)[6,7] in periodontitis.

Host-derived biomarkers associated with bone destruction:

Salivary biomarkers associated with bone destruction includes alkaline phosphatase, osteonectin, RANKL and salivary calcium. CAL has positive correlation with bone destruction. Salivary biomarkers and its correlations with periodontitis varies with stimulated and unstimulated samples over time. Higher levels of salivary MMP-8 in periodontitis were observed in non-stimulatory whole saliva.

Point of Care devices in periodontology:

A few Point of Care devices have been developed for the salivary diagnosis of periodontitis.

Integrated Microfluidic Platform for Oral Diagnosis

It is a device called the Integrated Microfluidic Platform for Oral Diagnosis.With this device, salivary proteins can be detected with low volume requirements (10μL) with electrophoretic immunoassays and laser – induced fluorescence detection system.Using this device rapid measurements within < 10 min of MMP-8, TNF-α, IL-6 and CRP in saliva were performed. Lab on a chip (LOC) system integrates microfluidics and a fluorescence based optical system in which sandwich immunoassay are performed on chemically sensitized beads.

LOC is developed by a group at the University of Texas at Austin. They reported the application of the LOC system for the multiplex measurement of three salivary biomarkers (MMP-8, IL-1β, C – reactive protein) that are related to the clinical expression of periodontitis and the results obtained by LOC were consistent with ELISA results. Using saliva, the success of Point of Care diagnostics of periodontitis study under large population were needed.

International Consortium for Salivary Biomarkers of Periodontitis(ICSBP) can put effort to create

1. standardized protocols for clinical research,
2. uniform method for clinical diagnoses of periodontitis.
3. can accelerate the validation of biomarkers

Requirements for saliva point of care platforms

Saliva is a useful diagnostic fluid for monitoring the body's health. Because of high mucin content it is very viscous compared with other body fluids.[8] Biological properties of saliva hinder its use in clinical application.In saliva, the biomarkers are usually dilute when compared with other body fluids. High sensitivity and high specificity detection is highly desired in salivary diagnostics with point of care method. The effect of variations in saliva content must be tested in a single patient under controlled conditions and under variable conditions to determine the specificity of the point of care test.

Accurate diagnosis requires multiplex detection of endogenous biomarkers and a single biomarker is not sufficient for an accurate diagnosis because of the complexity of biological system.Repeatability, intermediate precision and reproducibility determines the robustness of the point of care platform .For a clinical salivary diagnostics , the convenience and user friendliness of the device is a key factor , for untrained operators and resource – limited areas the Point-Of-Caredevice for saliva must be operated within a humidityrange of 10% to 90% and within a temperature range of4 to 30 °C.

Each of the Point-Of-Care devices contain a disposable part for each individual test. The price of the each is determined by the cost of the disposable part and the cost of the test will behigh where there is a requirement for specific reagent storageand processing. Because of simple structure, easy storage and low-cost. Lateral Force-Based Chromatography (test strip)has great potential.[9,10]

Development of low-cost Point-Of-Care platforms that can be integrated into healthcare delivery systems through information and communication technologies is a critical aspect of salivary diagnostics. With the integrated system, the operator simply collects saliva via the collector, introduces the salivary sample into the point-of-care platform. Later operates the software to control the device then waits for the results to be displayed on the instrument's screen. These remains a challenge, because providing the necessary connectivity of the analytical devices to clinical information systems and matching these devices to clinical need in order to facilitate their integration into the healthcare system.

Single biomarker-based point-of-care platforms for saliva

Point-of-care platforms have been developed for salivary diagnostics, bothfor oral and systemic diseases.µTASA Micro-Total analysis system Itis based onmicroelectromechanical technology. MMP-8 in salivacan be detected by a prototype µTAS developed by the Sandia National Lab. Thistest is based on an optical system with a fluorescencelabelledanti-MMP-8 (aMMP-8) antibody. These is followed byelectrophoresis.Using a 20 µl saliva samplethe detection is completed within 10minutes.And the results were compared with ELISA using a paired test.

Oral Risk Indicator

Dentognostics (Germany) has developed the Oral Risk Indicator® (ORI) platform for rapid aMMP-8 testing. Increased amount of aMMP-8 values suggest active inflammatory processes in the periodontium, such as gingivitis or periodontitis. Advantage of this platform is it identifies pathogenic concentrationsof the collagenase aMMP-8 in a mouth rinse specimen. The result is delivered in less than 10 minutes the test can be conducted by the medical secretary or nurse.

For systemic diseases, the development of saliva based prototypes is ongoing. OraSure Technologies Inc.has developed several lateral force-based chromatography test strips for rapid screening of infectious diseases using saliva. The assays include Human Immunodeficiency Virus, Hepatitis CVirus and influenza. Human Immunodeficiency Virus-1/2, HCV antibody and influenzaA/B testing can be performed in 20 minutes.[11]Healthcare practitioners easily identify those infected by this method due to its accurate results and easy application.

Point-of-care platforms for cortisol-related salivary testing have also been reported.[12] Hand held device developed byNipro (Japan) used to monitor the salivary a-amylase level and this helps to evaluate humanstress levels. This device requires 30 µl of saliva andthe measurement can be done in 1 minute with a coefficientof $R2 = 0.97$.

Conclusion

In summary continued research in this field are essential,numerous advantages are there in using Point of Care for salivary diagnostics which can improve patient compliance for testing the benefits of scalability, low cost, disposable device, the use of small sample volume,automated process and rapid process. Applying point-of-care platforms to salivary diagnostics will have impact on the healthcare system.Bioinformatics need to be introduced into the point-of-care system-generated salivary database.So, large amounts of medical information will be generated in of Point-of-Care.Data-sharing functions, and, the related data safety function should be included in point of care in future.

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