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An Approach Towards Salivary Biomarkers Of Periodontal Disease – "Marker To Cease Disease"

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ABSTRACT

Periodontal diseases are complex, multifactorial chronic inflammatory infections which affect the bone and soft tissue that support the teeth. Microorganisms and microbial products in the plaque biofilm are the main etiologic factors that lead to degradation of the supportive periodontal structures. In addition, the host immune response, as well as local and systemic factors, plays a significant role in the process of destruction or maintenance of the periodontium.Biomarker is the predictor for periodontal disease progression, diagnosis, treatment and prognosis.

Keywords: Periodontitis, biomarkers, oral diagnostic kits

I. Introduction

The term "biomarker" refers to a "parameter that is objectively tested and analyzed as an indicator of normal biological or pathological processes, or pharmacological responses to a therapeutic intervention" (NIH 1998).The goal of periodontal diagnostic procedures is to provide pertinent information to the clinician regarding the current periodontal disease type, location, severity, and serve as basis for treatment planning, providing essential data during periodontal maintenance and disease-monitoring phases of treatment. An ideal Periodontal diagnostic biomarker should be able to achieve goals such as to identify current disease activity, differentiate active sites from inactive ones, predict further disease progression and monitor the response to periodontal therapy.[1]An ideal biomarker must bespecific,sensitive, predictive, rapid, economical, non-invasive, stable, in vivo and in vitro.[2] The candidates for biomarkers in periodontal disease include[3]sub gingival bacteria and their products,host inflammatory and immune products,proteolytic and hydrolytic enzymes, enzymes released from dead cells and connective tissue degradation products.The categories of biomarkers include GCF biomarkers, Salivary biomarkers, Bone biomarkers, Proteomic biomarkers and Genomic biomarkers.

II. Gingival crevicular fluid (GCF) as a Biomarker

GCF is a physiological fluid as well as an inflammatory exudate that can be collected from the gingival crevice surrounding the teeth. GCF sampling methods have been shown to capture inflammatory and connective tissue breakdown mediators accurately. It has the potential function as a growth medium that can support growth of host cells serving the function of serum, or in support of proliferation of periodontopathogens.[3]

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III. Biomarkers that are released into GCF

The biomarkers released in GCF include the inflammatory mediators like cytokines, interleukins, tumor necrosis factor-α, interferon-α, prostaglandin e2, matrix metalloprotinase, transferrin, c-reactive protein. Host derived biomarkers include aspartate aminotransferase, elastase, cathepsin g, β-glucuronidase, alkaline phosphatase, acid phosphatase, myeloperoxidase. Connective tissue breakdown products include collagen-telopeptides, osteocalcin, proteoglycans, breakdown products, fibronectin fragments.[4]

IV. Gratification of Saliva over Gingival Crevicular Fluid

The advantages of saliva over collecting GCF include painless collection process, less distress to patient, simple to collect and non-invasive, convenient for multi sampling, cost effective for screening large population, cheap technology for laboratorial diagnosis, easy to store, less time consuming and less blood contamination.[5]

V. Saliva as a Biomarker

"Robust" biomarkers are defined as those salivary proteins which have been shown to discriminate between periodontitis and oral health in at least 3 cross-sectional studies (with comparatively little or no documented evidence to the contrary) and for which longitudinal studies exploring the natural course of periodontitis and/or the impact of therapy on biomarker levels may provide supportive evidence. "Potential" biomarkers are identified using identical criteria to "robust" biomarkers with the exception that there are 2 replicated cross-sectional studies showing disease discrimination in addition to possible supporting evidence from longitudinal studies but for which there may be limited contradictory studies. It is accepted that the entries in the "robust" and "potential" categories may be interchangeable depending on the existence of further studies which remain unpublished for commercial reasons. "Uncertain" biomarkers are proteins for which there are just a few studies demonstrating periodontitis discrimination or for which there are multiple studies with inconsistent results. In the lack of any evidence to the contrary, "unlikely" biomarkers are proteins for which three or more studies have failed to show evidence for a relationship with periodontitis.[6]

VI. Method of collecting saliva[7]

The methods of collecting saliva include Passive drool, Oral swab, Infant swab, Spitting method and Suction method.

VII. Specific markers

Immunoglobulins (Ig) are important specific defense factors of saliva. Of the different classes of immunoglobulins, IgA, IgG and IgM influence the oral microbiota by interfering with the adherence of bacteria or by inhibiting bacterial metabolism, with IgA being the predominant immunoglobulin. When compared to healthy patients, patients with periodontal disease had higher salivary concentrations of IgA, IgG, and IgM specific to periodontal pathogens. Levels of these immunoglobulins in saliva are greatly reduced after periodontal treatment in a study done by Reiff et al .[8] Screening of saliva, especially for IgA are useful, noninvasive technique

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to identify individuals who have the potential to develop periodontal disease or those who are currently responding to a periodontopathogenic infection.[9]

VII. Inflammatory markers

Prostaglandins are arachidonic acid metabolites that are divided into ten classes, the most important of which are D, E, F, G, H, and I. PGE2 acts as a potent vasodilator and increases capillary permeability, which elicits clinical signs of redness and edema. Airilla Mansson et al reported that PGE2 also stimulates fibroblasts and osteoclasts to increase the production of MMPs. MMP-8 is the most prevalent MMP found in diseased periodontal tissue and gingival crevicular fluid.[10] Elevated MMP-8 levels in active disease progression were observed in a longitudinal study of patients with gingivitis and with nonprogressive and progressive periodontitis in a study done by Birkedal Hansen H.[11]

VII. Blood components

Serum or plasma provides information about the inflammatory stimulus and/or response generated in circulation towards the periodontal pathogens that colonize in the subgingival area. The main disadvantage with blood sample are chances of cross – contamination as well as a potential risk to the health care personnel and more invasive than GCF or saliva.

VIII. Oral diagnostic kits[14]

Oral Fluid Nano Sensor Test (OFNASET)detects oral cancer in saliva by Dr. David Wong in 2012. This is an automated point-of-care device that detects various salivary proteins and nucleic acids using electrochemistry. It is an ultra-sensitive and ultraspecific micro electromechanical system which simultaneously and precisely detects these proteins and nucleic acid. Four salivary mRNA biomarkers (SAT, ODZ, IL-8 and IL-1b) and two salivary proteomic biomarkers (thioredoxin and IL-8) in saliva are detected in this system.Electronic taste chipswhich are alab-on-a-chip system, which will differentiate between healthy and periodontally diseased individuals, based on the CRP levels. This microchip-based detection system is used for measuring analytes (acids, bases, electrolytes and proteins) in solution phase. This novel system is called an Electronic Taste Chip (ETC). 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.OraQuickkitscreens and accurately diagnose HIV infection. Rapid POC HIV tests - provides results in 20 minutes. The fluid to be diagnosed is mixed in a vial with developing solution and the results are displayed on a testing device. It is a stick-like device with a fabric swab on one end which is inserted into a tube of testing fluid. OraQuick is the first FDA-approved oral swab in-home test for HIV-1 and HIV-2.Integrated microfluidic platform for oral diagnostics (IMPOD) helps in the rapid quantification of salivary biomarkers related to oral disease. Integrating sample pretreatment with electrophoretic immunoassays to swiftly assess analyte concentrations in lightly pretreated saliva samples allows for hands-free saliva analysis. Rapid measurement of levels MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved. The hand-held IMPOD has been used to rapidly (3–10 minutes) measure the concentrations of MMP-8 and other biomarkers

in small amounts (10 ml) of saliva. Level of MMP-8 was demonstrated to be highly elevated in saliva from patients with periodontal disease using a rapid point-of-care microfluidic device – Herr et al 2007.My PerioPathdetects 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.

Omnigene detects species specific DNA probes to identify eight pathogens which are known to cause periodontal disease, *(Porphyromonasgingivalis, Prevotella intermedia, Aggregatibacteractinomycetem-comitans, Fusobacterium nucleatum, Eikenellacorrodens, Campylobacter rectus, Bacteroides forsythus and Treponema denticola*). The advantage of this is that the results are available in short period of time and can be mailed or faxed to the clinician. The genetic test includePeriodontitis Susceptibility Trait test (PST) - genetic predisposition of the patient for periodontitis by detecting the polymorphism in IL-1 gene. Polymorphism in two positions of IL-1 that is position -889 and + 3953 has been associated with periodontal disease.MyperioID is a genetic susceptibility of the patient to periodontal diseases by using salivary samples which are shipped to the laboratory for the results. These test play role in evaluating the patients which are at higher risk of periodontal destruction.The Pocket Watch is a chairside test for AST levels analysis.

MMPs are host-derived proteinases which plays a major role in periodontitis and dental peri-implant health and diseases. Monoclonal antibodies for MMP-8 are being utilized in chair side POC immunotests for oral fluid and serum MMP-8 analysis. 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 (IFMA).

VIII. Recent advances

Point of care testing (POC) testing is defined as medical testing conducted outside of a laboratory at or near the site of patient care, including the patient's bedside, the doctor's office, and the patient's home (Song et al., 2014). The method uses oral fluid sampling and analysis with a rapid point-of-care or lab-on-a-chip device for the generation of a periodontal disease biomarker report.[16]

IX. Salivomics

A key advance in this area is the development of the salivomics knowledge database (SKB) and the salivary proteome knowledge base which is part of the human salivary proteome project. Salivomics is an emerging discipline which describes the study of related sets of biological molecules including the transcriptome, the proteome, and the metabolome in saliva and which, it is envisaged, will drive the development of personalized diagnostic approaches in the dental clinic. The SKB includes a data management facility that sources salivomics research data and connects to other databases.[17]

X. Salivary Transcriptome

The salivary transcriptome refers to a collection of transcripts, deoxyribonucleic acid (DNA) that is transcribed into ribonucleic acid (RNA), within saliva. Salivary transcriptomes (RNA

molecules) - are unusually stable in saliva. Second diagnostic alphabet in saliva and opened a door to another avenue of salivary transcriptomic diagnostics. Difference in gene expression between saliva from healthy and periodontal disease patients can be identified.[17]

XI. Proteomic studies

A number of preliminary studies investigating the proteomic profile of saliva in periodontitis using a combination of 2D electrophoresis and mass spectrometry have been reported. Although this approach determines the salivary protein profile in an unbiased analysis, the approach is only sensitive enough to detect proteins with a relatively high abundance and many mediators, for example, cytokines and MMPs, are not in sufficiently high concentrations to be detected. A comparative study of salivary proteins from 5 patients with generalised aggressive periodontitis (GAgP) and 5 healthy patients, 11 proteins were found to be altered in the GAgP patients; theseincluded high abundance proteins such as amylase, lactoferrin, IgG2, IgA2, and albumin, which have previously been linked to inflammation. Increased vitamin-D binding protein was also found to be associated with localized aggressive periodontitis (LAgp) for the first time.[18]

XII. Conclusion

A tremendous amount of research activity is currently under way to explore the role of oral fluids as a possible medium in a variety of applications. The evolutionary process has promoted the discovery of new biomarkers and the development of new therapeutic approaches mainly using host modulation. These recent advances are leading to the development of more powerful diagnostic tools for Periodontists to optimize their treatment predictability and success outcome. Future developments in proteomic analysis and personalized medicine will pave the way allowing novel diagnostic tools.

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