Review Article

Salivary biomarkers in the detection of periodontal diseases

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ABSTRACT

Periodontitis is a disease characterized by loss of connective tissue attachment and bone around the teeth in connection with the formation of periodontal pockets due to the apical migration of the junctional epithelium. Early diagnosis and treatment of progressive periodontitis are important because of the irreversible nature of this disease. Biochemical mediators in oral fluids like saliva are highly beneficial in the determination of current periodontal status. These substances known as biomarkers help in the determination of inflammatory mediator levels, as they are good indicators of inflammatory activity. This review is about the uses of biomarkers in the detection of periodontitis.

KEY WORDS: Periodontal diseases, saliva, biomarkers, detective aids

INTRODUCTION

Gingivitis and periodontitis are chronic inflammatory conditions that may affect as much as 80% of the adult population.[1,2] The disease process is initiated by the accumulation of bacteria along the gingival margin and in the interface between the gingival tissues and the teeth. Bacterial cells initially colonize the acquired salivary pellicle, and if their accumulation is not disturbed by oral hygiene practices, bacteria will grow and proliferate, giving rise to a complex structure currently termed bacterial biofilm.[3] As the biofilm develops and matures, bacterial succession occurs, which refers to the ability of other bacterial species than the ones initially colonizing the salivary pellicle to establish themselves within the extracellular polysaccharide matrix and the already attached bacteria.[4,5] Bacterial succession is responsible for a pathogenic shift in the gingival/periodontium, where the proportion of Gram-negative anaerobes tends to increase as the biofilm matures. Gingival tissues respond to the accumulation of bacteria and exposure to bacterial products with inflammation.[6] The inflammatory changes are initially confined to the soft tissues, involve clinical changes in volume, color, shape, position, and texture of the gingival tissues, and are often accompanied by bleeding on probing. The clinical condition is termed gingivitis, which is associated neither with apical migration of the junctional epithelium nor with destruction of bone and periodontal ligament fibers. Gingivitis is a reversible condition that can usually be treated with professional biofilm removal and improvement in oral hygiene. Most cases of long-standing gingivitis progress into periodontitis. Periodontitis is an inflammatory condition of the supporting structures of the teeth and involves attachment and bone loss. If left untreated, periodontitis may lead to tooth loss.[7] Periodontitis occurs because the inflammatory process migrates through the gingival tissues in the apical direction, following the paths of larger blood vessels. The inflammatory process contains numerous enzymes and cytokines, notably collagenases and prostaglandins, which degrade collagen and induce the activation of osteoclasts, resulting in attachment and bone loss.[8,9] Clinically, periodontitis shares signs and symptoms with gingivitis, but it differs from the latter by showing apical migration of the junctional epithelium from the cement-enamel junction, which results in increased pocket depth. As pockets increase in depth, biofilm removal becomes more difficult, leading to the development of more inflammation and tissue destruction that contributes to the perpetuation of periodontitis.

COMMON DIAGNOSTIC AIDS FOR PERIODONTAL DISEASES

The diagnosis of periodontal diseases is usually made by a dental practitioner, and it includes visual

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inspection of the gingival tissues, the measurement of pocket depth and attachment loss, the observation of bleeding on probing, and other clinical examination such as gingival recession, tooth mobility, and furcation invasions.[10] Radiographs are used as supporting information to the clinical findings as they reveal bone levels around teeth, besides providing other information about teeth and mineralized tissues. Inflamed gingival tissues often appear swollen with rolled borders, which are present with soft textures and redness in color. On the other hand, the presence of pain and fever which are often associated with the presence of inflammation in other areas of the human body is usually missing from the clinical picture associated with gingivitis and periodontitis.[11] The absence of pain in periodontal diseases is usually one of the main reasons why patients do not seek professional care in their early or even more advanced stages. This is particularly true for the segment of the population who does not receive dental care regularly in the form of periodic examinations and preventive professional dental recalls. Patients may not be aware of and/or choose to ignore the signs and symptoms of gingival and periodontal inflammation such as red and bleeding gums, thus allowing the disease process to progress to a point at which it may require extensive periodontal treatment or where tooth loss can no longer be prevented.

SALIVARY DIAGNOSTICS AIDS

Studies reveal a promising outlook for saliva as a key diagnostic medium for determining systemic diseases or health statuses of individuals.[12,13] As collecting saliva involves non-invasive methods, and due to the fact that it is an abundant and easily accessible biofluid, saliva is attractive for diagnostic purposes greatly due to its highly enriched content of disease biomarkers that can be deciphered and analyzed. Biomarkers for the detection of specific diseases, such as Sjögren's syndrome, pancreatic, breast, and oral cancer, or periodontal diseases can be detected in saliva.[14,15] These properties of saliva open doors to a perfect method of exploring health and disease surveillance in clinical settings with just a minute amount of the oral fluid.

SALIVARY DIAGNOSTIC AIDS FOR PERIODONTAL DISEASES

There are currently two salivary diagnostic tests available in the American market for the detection of periodontal diseases.[16] These tests enable clinicians to collect saliva and send the samples to a laboratory, where deoxyribonucleic acid (DNA)-polymerase chain reaction analysis is used to complete the diagnostic tests and devise risk assessment reports. One test identifies the type and concentration of specific periodontal pathogenic microorganisms in patients' saliva samples. Another salivary test claims to detect genetic susceptibility to periodontitis in individuals, which allows the clinician to identify patients who are at a greater risk for the development of severe periodontal destruction.[16] Both salivary tests state that they can potentially detect the periodontal diseases early and evaluate the susceptibility to periodontitis in individuals. Although the tests claim to be quick and easily administered at chair-side, 4-5 days are required for the laboratory results to be returned to the clinician. These tests identify general risk factors for the development of periodontitis but lack the ability to determine when periodontal destruction will occur, thereby not being able to specifically predict periods of disease activity.[16]

A salivary occult blood test (SOBT), which has been proposed as a periodontal status screening method, is currently available in Japan.[17] This method has been reported to detect individuals with poor periodontal health, which is defined as bleeding on probing in ≥20% of teeth or the presence of probing pocket depth ≥6 mm plus bleeding on probing in ≥1 teeth. A proprietary paper strip containing gold-labeled anti-human hemoglobin monoclonal antibody is dipped into the saliva sample. On forming an immune complex with hemoglobin, the immune complex travels up the paper strip by capillary action until it is immobilized. This results into a magenta line, indicating a positive test result for the manufacturer's reference concentration of ≥2 μg/ml human hemoglobin. A study of 1998 subjects in a suburb of the Fukuoka metropolitan area in southern Japan reported that sensitivity and specificity of the SOBT in screening for poor periodontal status were 0.72 and 0.52, respectively.[17] The investigators involved in this study suggested that these values were not very high; however, SOBT can be utilized as a simple screening method at a low cost for identifying periodontal status of a patients and increasing their oral health awareness. The salivary diagnostic tests mentioned above claim to detect periodontal diseases based on purely microbial or single inflammatory-based information. They could be labeled as first-generation tests involving saliva in the diagnosis and prognosis of patients with periodontal diseases, and they represent the beginning of an era where disease-associated agents are detected and analyzed in saliva. Giannobile is leading the field by combining microbial biomarkers from periodontal pathogens and salivary biomarkers from host-response changes to better understand the multifactorial nature of periodontal diseases.[18,19] Since the existing tests do not allow for a direct correlation between the presence of specific bacteria and isolated inflammatory markers with the predictive value of periodontal attachment and bone loss, the next generation of salivary tests is presumed to be enhanced by enabling the understanding of these relationships in the diagnosis and prognosis of periodontal diseases.

DIAGNOSITIC ADVANCEMENT FOR THE DETECTION OF PERIODONTAL DISEASES USING SALIVA

Even though the diagnostic value of saliva has been recognized and several potential biomarkers of periodontal diseases identified, most of the work conducted to date came short of providing clinically reliable and useful information for practitioners in terms of developing a more precise periodontal diagnosis and subsequent treatment planning.[20,21] In order for the salivary diagnostics for periodontal diseases to be clinically relevant, the appropriate bioinformatics has to augment biomarker discovery so that validated biomarkers have disease discriminatory power. The test also needs to be real time, where the patient's periodontal status can be immediately evaluated while he/she is in the dental office. Finally, biomarkers should not only diagnose the disease but also predict the risk of future disease activity by simple and affordable means. Recent advances in transcriptomic high-throughput technologies are shedding new light on salivary biomarker discovery, which can elevate salivary diagnosis of periodontal diseases to a higher level. The salivary transcriptome refers to a collection of transcripts, DNA is transcribed into ribonucleic acid (RNA), within saliva. By analyzing the transcriptome, one can assess which genes are turned on or off and if there is a difference in gene expression between saliva from healthy and periodontal-diseased patients. Wong at UCLA believe that biomarkers found in saliva may actually predict bursts of periodontal disease activity. They are in the process of discovering gene signatures in patients by performing multiplex transcriptomic analysis of messenger RNA in human saliva. The basis for this investigation in the field of periodontal diseases is derived from a similar approach that has been successfully applied to the detection of cancer-associated biomarkers in saliva. In fact, it has been proved that saliva contains disease discriminatory mRNAs in oral cancer. Using high-density oligonucleotide microarrays (54,000 probe sets representing approximately 38,500 genes), saliva mRNAs from healthy and oral cancer patients have been thoroughly profiled and statically compared. They discovered four mRNA biomarkers for oral cancer with a sensitivity of 91% and specificity of 91% to distinguish cancer from the normal.[22,23] The same discovery platform can be used to profile healthy and periodontal transcriptomes and to find the most significant candidate genes for the onset and progression of periodontal diseases. Those discriminatory candidate genes must be validated for their sensitivity and specificity as saliva biomarkers. In addition to technological advances, in order for salivary biomarkers for periodontal diseases to have the intended clinical context, study design and clinical trials must reflect the ultimate goal of obtaining approval from the Food and Drug Administration (FDA) agency. Recently, the FDA approved the oral fluid-based human immunodeficiency virus antibody test. This milestone achievement in salivary diagnostics proved that saliva can be a disease-discriminatory biofluid much like the "gold-standard" serum. Second, it reminded the research community that the end goal of future salivary biomarker discovery is the application of novel treatment and therapeutics to the real world by translating the knowledge from the laboratory bench to a medical or dental setting. Thus, the study design and clinical trials for the biomarker discovery should minimize bias and maximize clinically relevance. Clinical trial design such as the Prospective Randomized Open Blinded End-Point design maximizes similarity to standard clinical practice, makes the research results more easily applicable in routine dental settings, and aids in the translation step of future biomarker discovery.

CONCLUSION

Periodontal disease diagnosis and follow-up care will greatly advance in the near future through the discovery of disease-specific salivary biomarkers. Prototype electrochemical devices such as the oral fluid periodontal health test may provide accurate and real-time assessments of periodontal diseases for the general public either at home or at the dental office. Although challenges remain ahead, using saliva to gauge periodontal health appears bright for future application to aid in the diagnosis of periodontal diseases and the prediction of periodontal treatment outcomes.

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