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Review Article

Salivaomics - A promising future in early diagnosis of dental diseases

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ABSTRACT

Human saliva plays an important role in the health of the oral cavity and of the body as a whole. Salivary diagnostics is a dynamic and emerging field in the diagnosis of oral and systemic diseases. Saliva reflects the physiologic state of the body, including emotional, endocrinal, nutritional, and metabolic variations. The collection of saliva samples is noninvasive, safe, and inexpensive. Traditional clinical criteria are insufficient for determining sites of active disease, for monitoring the response to therapy, or for measuring the degree of susceptibility to future disease progression. Salivaomics includes five diagnostic alphabets proteins, mRNAs, miRNAs, metabolic compounds, and microbes offering substantial advantages because disease states may be accompanied by detectable changes. Salivaomics, the future of saliva-based techniques for early diagnosis of dental diseases, is promising and may offer a robust alternative for clinicians to use in the near future to make clinical decisions.

Key Words: Biomarker, microbiome, proteome, saliva, transcriptome

INTRODUCTION

Periodontal diseases, dental caries, malocclusion, and oral cancer are the major dental problems affecting people worldwide as well as in Indian community. Periodontal diseases include a group of chronic inflammatory diseases that affect the periodontal supporting tissues of teeth and encompass destructive and nondestructive diseases. Various samples available for diagnosis of periodontal diseases include gingival crevicular fluid, plaque, saliva, biopsies, peripheral blood cells, and plasma.[1]

The identification of susceptible individuals or sites at risk from disease, and the diagnosis of active phases of periodontal disease, represents a challenge for both clinicians and oral health researchers.[1] Currently, diagnosis of periodontal disease relies primarily on clinical and radiographic parameters. These measures are useful in detecting evidence of past disease or verifying periodontal health, but provide only limited information about patients and sites at risk for future periodontal breakdown.[2]

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Ideally, diagnostic tests should demonstrate high specificity and sensitivity. Numerous markers in saliva have been proposed and used as diagnostic tests for periodontal disease.[2] Given the complex nature of periodontal disease, it is unlikely that a single marker will prove to be both sensitive and specific. A combination of two or more markers may provide a more accurate assessment of the periodontal patient.[3] A plethora of biomarkers and diagnostic tests were developed, several of which demonstrated high levels of sensitivity, specificity, and diagnostic accuracy with respect to identifying and/or predicting disease activity at the site level. Three of the most promising biomarkers for predicting future disease activity were beta-glucuronidase (78% diagnostic accuracy), alkaline phosphatase (77% diagnostic accuracy), and cathepsin B (99% diagnostic accuracy).[4] A number of diagnostic kits emerged based upon individual biomarkers within gingival crevicular fluid, but market research had not been performed and the tests failed to show significant impact upon the practicing community for several reasons like the results did not improve any therapeutic interventions.[5]

Early detection of disease or risk of disease should be the main aim of a diagnostic test, allowing to minimize the dynamics of progression of disease or to prevent the disease. Traditional clinical criteria are insufficient for determining sites of active disease, for monitoring the response to therapy, or for measuring the degree of susceptibility to future disease progression. Limitations in recognizing the full potential of disease detection are lack of an easy and inexpensive sampling method with minimal discomfort, and lack of an accurate, easy-to-use, and portable platform to facilitate early disease detection.[5,6]

Though saliva has enormous elements with diagnostic potential, omic technology made it possible to achieve the best of the saliva's diagnostic potential into the clinical practice. The aim of the present review is to update information on the use of salivaomic technology for early diagnosis of dental diseases. A literature search of Medline, Cochrane Library, and Google Scholar was performed using the following search terms: "Saliva", "proteome", "microbiome", "transcriptome" and "metabolome."

Inclusion criteria

Articles currently available on salivaomic technologies with regard to dental research were taken into consideration.

Exclusion criteria

Studies on omic technologies with gingival crevicular fluid, plaque, biopsies, peripheral blood cells, and plasma samples were not considered in this review.

Saliva

Saliva has a complex secretion of salivary glands which constantly bathes the teeth and oral mucosa. Saliva performs several key functions, including protection of the oral cavity from infections such as caries and promotes swallowing and degradation of ingested food. Saliva also affects taste sensation.[7] Important functions of saliva with respect to the teeth include buffering action by means of bicarbonate and phosphates in proteins, lubrication, remineralization of enamel by the proline-rich proteins, statherin, calcium, phosphates, and inhibition of demineralization associated with mucins. In general, antibacterial, antifungal, and antiviral activities are associated with the mucins, lactoferrin, lactoperoxidase, histatins, cystatins, and immunoglobulins found in saliva.[8] Diagnosis of active phase of periodontal disease and the identification of the patients at risk for active disease represents a challenge for both clinicians and clinical investigators. Saliva is a fluid that can be easily collected and contains locally derived and systemically derived markers of oral disease.[9]

Saliva as a mirror of oral and systemic health is a valuable source for clinically relevant information, because it contains biomarkers specific for the unique physiological aspects of periodontal diseases.[8,9] Many clinically important molecules can be detected through salivary testing, including salivary constituents that have been studied as potential diagnostic biomarkers for periodontal disease mainly include locally produced proteins of host and bacterial origin (enzymes, immunoglobulins, and cytokines), genetic/genomic biomarkers such as DNA and mRNA of host origin, bacteria and bacterial products, ions, steroid hormones, and volatile compounds.[10]

Salivary markers of periodontal diseases

A biomarker should aid in early detection of disease, prognosis of disease outcome and possible patient stratification allowing personalized medical interventions, prediction of treatment outcome, identification of patients who will respond well to a particular treatment, and surrogate end-point.[11,12]

To date, there is no single biomarker that is specific for periodontal disease. Therefore, there is strong potential for the use of microbial and host-response biomarkers in combination to enhance identification of the disease process, given the multifactorial nature of periodontal diseases.[13]

The development of chair-side point of care devices for oral health surveillance likely will require minimal clinical training and resources may lead to better use by properly trained practitioners for simpler and less intensive treatment and likely will result in more cost-effective oral health care delivery.[14]

Dental caries

Salivary tests have recently been developed to evaluate caries risk by measuring the amounts of selected oligosaccharides whose concentrations have shown a correlation with caries experience in young adults. In a study, authors used a proteomic approach to test whether salivary proteins can act as biomarkers for caries risk assessment.[14] Their data suggest that statherin and cystatins in saliva are the best predictors of occlusal caries. It is possible that these findings suggest a relationship between the molecular functions of statherin and cystatins and the antimicrobial properties of saliva.[15]

Using the emerging omic technologies, salivary proteins and RNAs can be used to detect cancer and Sjogren's syndrome. The emerging omic technology offers highly sensitive and specific, economical as ideal diagnostic test.[16]

Omic technology

Omic technologies include genomics, transcriptomics, proteomics, and metabolomics. The achievements of high-through put approaches such as proteomics including free-flow electrophoresis coupled with linear ion-trap tandem mass spectrometry, multidimensional separation platform based on nano-reversed phase liquid chromatography, and capillary isoelectric focusing, two dimensional gel electrophoresis coupled with matrix-assisted laser desorption/ionization time of flight mass spectrometry or liquid chromatography-mass spectrometry, Isobaric tag for relative and absolute quantitation and label-free quantitation, microarray and microfluidics afforded by modern diagnostic techniques allow for disease-specific salivary biomarker discovery and establishment of multiplex, rapid, and miniaturized analytical salivary assays.[15-20]

Salivaomics

The term "salivaomics" was coined in 2008 to reflect the rapid development of knowledge about the various "omics" constituents of saliva. Salivaomics [Figure 1] includes five diagnostic alphabets proteins, mRNAs, miRNAs, metabolic compounds, and microbes offers substantial advantages because disease states may be accompanied by detectable changes in one, but not all, dimensions.[21]

Genomics is the study of whole genomes, that is, all the DNA of a single organism. With improvements in sequencing, the dawn of genome-driven individualized medicine has arrived, where changes to multiple genes may be taken into account for diagnosis and treatment.[20] Most relevant to periodontal diseases are the emerging toolboxes of the salivary proteome and the salivary transcriptome for early detection, disease progression, and therapeutic monitoring.

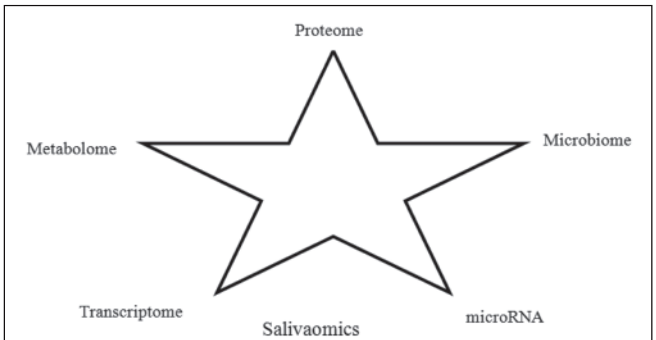

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Figure 1: Salivaomics

Salivary proteomics

Proteomics is the study of all the proteins in a given sample.[21] Human salivary proteome analysis is important for understanding oral health and disease pathogenesis. Protein expression is primarily analyzed by one- or two-dimensional polyacrylamide gel electrophoresis (PAGE). To resolve the complex composition of saliva, two-dimensional PAGE allows separation not only of different molecules with similar molecular weights, but also of different modification patterns or isoforms of the same protein.[22] By using both two-dimensional gel electrophoresis/ mass spectrometry and shotgun proteomics approaches, it has been identified 309 distinct proteins in human whole saliva. A total of 1,166 salivary proteins have been identified-914 from the parotid fluid and 917 from the combined submandibular and sublingual fluids.[23]

In the study of periodontal diseases, many proteomic approaches have been used. A study was conducted in patients with periodontitis (n = 10) in comparison to control (n = 4), with the samples of gingival crevicular fluid, serum and saliva by electrophoresis, and mass spectrometry S100A8 and S100A9 represented major differences between gingival crevicular fluid and saliva.[24] Another study was done in aggressive periodontitis patients with salivary samples resulted in six proteins were increased in saliva of periodontitis subjects, while five were decreased.[25]

Salivary metabolomics

Metabolomics is the global assessment and validation of endogenous small-molecule metabolites within a biologic system that has gained increasing popularity and significance in life sciences.[26] Analysis of these key metabolites in body fluids has become an important role to monitor the state of biological organisms and is a widely used diagnostic tool for disease. Metabolomics provides potential advantages that classical diagnostic approaches do not, based on the discovery of clinically relevant biomarkers that are affected by the disease.[27]

Saliva-based diagnostics, particularly, those based on metabolomic technology, are emerging and offer a promising clinical strategy, characterizing the association between salivary analytes and a particular disease. To date, the development and application of analytical technology for the detailed analysis of saliva has led to the discovery of numerous disease biomarkers and could enable separation, detection, characterization, and quantification of the saliva metabolome.

Salivary transcriptome

High-density oligonucleotide microarrays were used to profile salivary mRNA and revealed that there are 3,000 human mRNAs in the cell-free saliva supernatant of healthy subjects. Of particular interest is that there is a normal saliva transcriptome core signature of 185 mRNAs that is present in all normal subjects, providing the rationale to use the salivary transcriptome for disease detection.[14,28]

The salivary microbiome is a promising clinical diagnostic indicator of oral cancer, periodontitis, and possibly other diseases. The presence of specific pathogens and disturbed oral bacterial community might indicate the disease before symptoms are evident and may have clinical applications.[29]

The research team at *University of California, Los Angeles (*ULCA) developed Salivaomics Knowledge Base (SKB), a data management system and web resource that supports salivary diagnostics research to overcome the inability to cross-reference data sets from different types of studies.[21]

At UCLA, the research team is building SKB on the basis of the saliva ontology (SALO) and SDxMart, which allow the SKB to operate with other omics databases to facilitate integration of heterogeneous and disparate data sources in a systems biology approach. SALO and SDxMart are the first of their kind in dentistry.[21]

The SALO is a detailed ontology of saliva that is optimized to meet the needs of both the clinical diagnostic community and the cross-disciplinary community of omics researchers. The SDxMart is a BioMart data portal that hosts salivary proteomic, transcriptomic, metabolomic, and miRNA data and offers access to the data via use of the BioMart interface and querying environment.

Point-of-care technologies for salivary diagnostics

Application of microfluidics and micro/nano electro mechanical system (MEMS/NEMS) to saliva-based diagnostics provides good point of care diagnosis. MEMS/NEMS is an integrated system that consists of a central unit for processing data and several other components that connect with the outside interface such as microsensors.[30] Multiple analytes in a drop of saliva could be simultaneously measured and analyzed through highly sensitive biosensors. Oral fluid nanosensor test is a point-of-care, automated, and easy-to-use integrated system that will enable simultaneous and precise detection of multiple salivary proteins and nucleic acids. This system is portable and could be used not only in the dental clinics, but also in any other hospitals to perform an instant point-of-care diagnosis.[31]

DISCUSSION

Diagnostic tests are routinely used in evaluation of many systemic disorders. Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Saliva meets the demands for an inexpensive, noninvasive, and easy-to-use diagnostic platform. The advantages of easy collection, storage, shipping, and voluminous sampling make saliva a better diagnostic biofluid than serum or urine. It is also easier to handle saliva during diagnostic procedures than blood, because it does not clot and this reduces the number of manipulations required.

Numerous markers in saliva have been proposed as diagnostic tests for periodontal disease. Composition of saliva is influenced directly or indirectly by most of the systemic diseases. Many researches in this field have enabled us to bridge oral health research with systemic disease diagnosis. Early detection, diagnosis, and assessment of prognosis of systemic diseases could be possible by monitoring the composition of saliva. Salivary diagnostics would enable clinicians to monitor diseases frequently and easily and would have impact on the future medical research and therapy.

Recently, due to the combination of emerging biotechnologies and salivary diagnostics, a large number of medically valuable analytes in saliva are gradually unveiled and some of them represent biomarkers for different diseases including cancer, autoimmune diseases, viral diseases, bacterial diseases, cardiovascular diseases, and HIV. These developments have extended the range of saliva-based diagnostics from the simple oral cavity to the whole physiological system. Genomic testing could allow risk-based long-term planning for more effective dental disease prevention, reduce the uncertainty of diagnosis and prognosis, and guide the selection of drugs or treatment protocols that minimize harmful side effects to ensure a more successful outcome for patients. Thus, saliva-based diagnostics is on the cutting edge of diagnostic technology and may offer a robust alternative for clinicians to use in the near future to make clinical decisions and predict post treatment outcomes.

CONCLUSION

Saliva is sound for detection of oral diseases like periodontal disease, caries, oral cancer, salivary gland disorders, and nonoral distal diseases. Though saliva has various markers to identify oral diseases, their ability for earlier detection before clinical signs and symptoms is questionable. Salivaomics, the future of saliva-based techniques for early diagnosis of dental diseases, is promising. However, as with any new technology, process issues need to be addressed before they are used widely in clinical settings.

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