Review Article

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Salivaomics : Emerging Era of Diagnosis

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ABSTRACT

Background: "Omics" technologies have contributed significantly in identification of alterations in gene expressions, transcription, protein coding and molecular identification. Salivaomics has integrated these technologies in saliva analysis presenting a novel approach in oral and some systemic disease diagnosis, prognosis and monitoring. Currently use of saliva in various diagnosis and research field is advancing due to use of various novel approaches including salivary microRNA (miRNA), salivary microbiome, metabolomics, genomics, proteomics and bioinformatics. Due to its non invasive collection and proximity to oral cancer site, salivary screening can be best choice for early detection of oral squamous cell carcinoma. Saliva has other distinctive advantage over blood serum is cost effective and anyone can collect it with modest training in large population. Analysis of saliva may be useful for diagnosis of hereditary, autoimmune, endocrine, infectious and malignant diseases. It's also useful in assessment of therapeutic levels of drugs and monitoring of forbidden drugs. Although, at present no single marker is helpful, a panel of biomarkers would be more appropriate for validating the presence of prognosis of the disease.

Keywords: Saliva, Biomarker, Proteomics, Microbiome, Metabolomics.

INTRODUCTION

In health care promotion, ability to monitor health, prevention and early detection of diseases through non-invasive means is highly desirable. Saliva is identified as a "Mirror of the Body". It is an emerging and in some cases proven medium for surveillance of oral and systemic diseases.

To diagnose oral or systemic disease, salivary tests were not used till 2012. To use saliva as strong medium of diagnosis it was required,

1. To develop and optimize diagnostic tools specifically for saliva
2. Need to have substantive scientific knowledge of salivary biomarkers reflecting systemic disease.

In recent developments, salivary constitute made it better suited for disease screening and risk assessment as it is non invasive, non expensive and easily mastered test methods. It can also facilitate precision medicine therapy by providing targeted, individualised, precise, pain free, and convenient therapy.

Recently significant advance work has been done in the development of salivary diagnostic tools. In future salivary diagnostics will have access to health care sector globally. Dentist will be able to screen various medical conditions through chair side screening systems. As for example, in case of early and fast detection of oral squamous cell carcinoma, salivary molecular markers have been identified in 3 different levels which includes

1. Changes occurring in the cellular DNA,
2. Alteration in mRNA transcripts and
3. Altered protein levels (intracellualrly, on cell surface and extracellularly) [(1)].

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As saliva is easy to collect, convenient to store, non invasive and contains high quality DNA, it can be considered a perfect substitute for blood or serum. Salivaomics research has an important role in identifying various biomarkers of diseases and many drug targets. It can also diagnose some diseases in early stages. The technique known as "liquid biopsy" enables detection of circulating tumour cells and fragments of tumour DNA in saliva. The new technique called electric field induced release and measurement provides near perfect results in detection of actionable mutations in lung cancer patients [2]. The translation of salivary biomarker reflection related to systemic diseases is progressing. Systematic knowledge of same with precise information of biomarkers related to diseases can contribute to a better understanding of correlation between oral and systemic health and widened salivary diagnostic approach from oral cavity to whole body. The purpose of this review is to update our knowledge regarding present and future application of saliva biofluid in different field of science.

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[3].

Though saliva has numerous elements with diagnostic potential, omic technology made it possible to achieve best of its potential in field of diagnosis on day to day practice. The disease related sets of biological molecules or "omics" constituents of saliva were studied and researched since 2008 and new era and branch of diagnosis was started termed as "Salivaomics". However, still it is in its early stage and progress is limited due to technical limitations and efficient methodology. So, there is a wide spread requirement of salivary molecular identification and standardised evaluation system. In ten years, new terms entered in the scientific lexicon. It includes diagnostic proteins, mRNAs, miRNAs, metabolic compounds, and microbes, which offers substantial advantages because disease states may be accompanied by detectable changes in one, but not all, dimensions[4]. So, in this review we briefly describe all components of Salivaomics like

1. *Salivary proteome,*[5]
2. *Salivary Transcriptome*
3. *Salivary microRNA (miRNA),*
4. *Salivary metabolome* [6] and
5. *Salivary microbiome*.

1. **Salivary proteome**

Proteomics is the study of all protein present within the given salivary sample. Human salivary proteome is important for understanding of oral health and disease. Protein analysis is primarily analyzed by one or two dimensional polyacrylamide gel electrophoresis (PAGE). To resolve the composition of saliva two dimensional PAGE allows separation of not only different molecules of same molecular weight, but also of different isoforms of the same protein. National Institute of Dental and Craniofacial Research (NIDCR)/National Institutes of Health (NIH) gave funding to three different groups for extensive research in the field of human salivary proteome. Research progressed in cataloguing human salivary proteins and explored their post translational modifications. Two dimensional gel electrophoresis/mass spectrometry and shotgun proteomics approaches identified 309 distinct proteins in human whole saliva [7]. After the said research Denny P et al[8] completed profile of salivary secretory proteome and identified 1,166 salivary proteins. Out of which, 914 were identified from parotid fluid and 917 from submandibular and sublingual fluids[8]. The University of California is the data centralisation centre, having database of human salivary proteome and termed as *Salivary Proteome Knowledge Base.*

2. Salivary transcriptome

The RNA molecules are elevated in cases of oral cancer in saliva, which prompted to explore the scope and complexity of RNA present in saliva (9,10). Cell free saliva of healthy subjects revealed 3000 mRNA by high density oligonucleotide microarrays. Out of which, normal saliva transcriptome core signature of 185 mRNA is present in all normal subjects providing rational for disease detection. It is shown that this endogenous mRNA is protected from degradation in a manner similar to cell free RNA in plasma [11,12].

Forensic sciences are focusing on multiplex of mRNA profiling for identifying different body fluids

including saliva [13, 14]. Salivary proteome and transcriptome are recent toolboxes for detection of salivary proteins and mRNA in detecting oral cancer [10, 15, 16,17] and sjogren's syndrome[18]. It is also envisioned that proteome tool can be used to identify markers for early detection, disease progression and monitoring therapy in patients with periodontal diseases.

3. Salivary Micro RNA

For many years it was thought that RNA can be degraded quickly by RNAses present in saliva [19]. Despite some reports [20], cell free RNA molecules seem to exist in the saliva in both fragmented as well as intact form [21]. So the question is how mRNA in saliva remains protected by degradation process? One speculation is such that salivary mRNA is contained in apoptopic bodies [22, 23] or actively released in exosomes or microvesicles [24-26]. So the microRNAs, small RNA molecules seem to regulate transcription were also discovered in saliva[27-29].

In forensic medicine also mRNA detection in saliva has been reported [14,15, 30, 31]. mRNA markers have also been proposed for the diagnosis of primary sjogren's syndrome [32] and identification of sleep drive both in flies and humans[33].

Similarly Li et al stated that various mRNA molecules were found to be upregulated in patients suffering from oral squamous cell carcinoma[10]. Some such molecules with their role are[34]

1. IL8 (Interleukin 8)- role in angiogenesis, replication, calcium mediated signalling, cell adhesion, chemotaxis, cell cycle arrest, immune response.
2. IL1β (Interleukin 1β)- signal transduction, inflammation, proliferation and apoptosis.
3. DUSP1(Dual specificity phophatase 1)- protein modification and oxidative stress.
4. H3F3A(H3 histone family 3A) – DNA binding activity
5. OAZ1(Ornithine dacarboxylase antizyme 1) – polyamine biosynthesis.
6. S100P(S100 calcium binding protein P)- protein binding and calcium binding.
7. SAT (spermidine/spermine N1-acetyltransferase) – enzyme and transferase activity.

4. Salivary metabolome

The word metabolome means complete set of small molecular metabolites found within a biological sample. 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. It may include metabolic intermediates in carbohydrate, lipid, amino acid, nucleic acid and other pathways. It also includes hormones and other signalling molecules. These diagnostic alphabets of saliva have positioned saliva in translational and clinical application and also in personalized medicine and dentistry.

What is Metabolomics?

It's a new form of omics research. Living cells contains metabolites derived from different metabolic activities like gene transcription, mRNA translation, enzymatic reactions and protein synthesis. Concluding, the comprehensive identification and quantification of these metabolites is called "Metabolomics".

Previously it was difficult to analyse numerous metabolites simultaneously and comprehensively, specifically small iconic metabolites e.g. phosphorylated sugars, carbonic acids and amino acids. In the past decade techniques like capillary electrophoresis (CE) and time of flight mass spectrometer (TOFMS), are developed which identify metabolites significantly. The CE is having excellent separation ability and can separate them by a slight difference of electric charge, as compared to liquid chromatography which cannot separate the metabolites having same mass. Coming to application of TOFMS in oral cancer research, in recent studies [35-40] cancer tissue is investigated using CE-TOFMS based metabolomics approach.

Ogawa et al. in 2014 performed metabolomics analysis of OSCC tissue using CE-TOFMS, and identified their metabolic systems[41]. Significant differences in some of the metabolites were detected between OSCC and normal tissues e.g. OSCC tissue showed decreased level of glucose and glutamine and increased lactate suggesting glucose consumption, metabolism of glutamine and production of lactate.

Concluding, metabolomics research is still in its early stages, but revealed several novel findings about different properties of metabolisms. In oral cancer research, it offered various insights, for example specific cancer metabolic pathways. In the future, analyses of oral biofilm, tissue and saliva, it might provide a range of novel information, accurate diagnosis, safe and effective treatment, and preventive measures for various diseases.

5. Salivary Microbiome

Collective genomes of the microbes that live inside and on the human body are called as human microbiome. It composed of bacteria, bacteriophages, fungi, protozoa, and viruses. Each human cell and genes are having 10 microbes and 100 microbial genes respectively.

In disease condition there is a shift in dynamic equilibrium away from the synergistic interplay towards the antagonistic interplay with colonisation of pathologic populations coupled with inflammatory host responses.

Epidemiological studies have reported an association of periodontal diseases and tooth loss with cancer [42, 43]. There is strong data to support such association with oral, oesophageal, gastric, and pancreatic cancer even after controlling the confounding factors like tobacco use[43]. Recently Porphyromonas Gingivalis, the periodontal pathogen has been identified as a biomarker for orodigestive tract cancer death [44]. There is also some evidence to support association between oral viral and fungal infection and cancer, as Human Papillomavirus-16 is one of the established cause of oropharyngeal squamous cell carcinoma [45]. Findings suggest that the upper digestive tract microbiota may play a role in the etiology of chronic atrophic gastritis and esophageal gastric dysplagia, and therefore in the development of gastric and esophageal cancers.

Early studies concluded that compared to normal tissue oral squamous cell carcinoma have significantly increase number of both aerobic and anaerobic bacteria in culture dependant assays. In addition 30% of sites were shown to harbour Candida Albicans also .[46]

In one pilot study using culture independent assays denaturing gradient gel electrophoresis, the microbiome in a series of 10 oral, tongue or floor of mouth cancers was compared to normal tissue in same patients. Streptococcus Intermedius was present in 70% of both cancer and normal sites. Streptococcus Gordonii, Streptococcus sp. oral taxon 058, Peptostreptococcus Stomatitis, Streptococcus Salivarius, Gamella Haemolysans, Gamella Morbillorum, Johnsonell Ignava and Streptococcus Parasanguinis were also associated with cancer and Granulicatella Adiacens was commonly present in the normal tissue(47).

So the technological advances have highlighted the structure of oral microbiome in health as well as disease. Further research is required to explore the oral microbiome for diagnosis and risk assessment of disease, as well as to decide therapeutic path to restore the health of the oral cavity.

CONCLUSION

Omics technologies have contributed significantly in the identification of alterations in gene expression, transcription, protein coding and small molecules concentration, in biologic systems. In this aspect, salivaomics integrate these technologies in saliva analysis and represent a novel and holistic approach in oral disease management including diagnosis, prognosis and monitoring. As we all know and believe since long that prevention is better, cheaper and smarter than cure. In India oral cancer is major health challenge for scientists, clinicians and policy makers. Research in the field of Salivaomics has an important role in identifying biomarkers of various oral and systemic diseases as well as early treatment plan. We need to develop techniques which are efficient, easy and sensitive to molecular identification for early detection of cancer. Saliva is novel body fluid for pain free, convenient collection and molecular cell detection, so one can use it in large group of population for early detection. Ongoing development of such methods will have profound impact on oral cancer screening and the early diagnosis of same, potentially resulting in early treatment and a decrease in the high levels of morbidity and mortality associated with OSCC. Furthermore, there are indications that, it may be possible to utilize our enhanced understanding of the chemokines

associated with disease progression, metastases, and bone invasion to develop novel methods for the treatment of oral cancer. This review summarizes the current research in the discovery of biomarkers and molecular signatures with diagnostic or prognostic utility for oral diseases in saliva. The review also focuses on the emerging issues of the salivaomics technology and saliva diagnostics and the translational potential.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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