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# Introductory Chapter: Saliva - The Future of Disease Diagnostics

*Gokul Sridharan*

## 1. Introduction

Saliva is a multi-constituent oral fluid and is considered a mirror of the body's health. The science of saliva has advanced exponentially in the past decade. Saliva and its constituents are being increasingly used for surveillance in various human diseases. The ability to assess the role of salivary components as potential biomarkers is a recent development with increased interest in its use as a diagnostic and prognostic tool [1]. The availability of newer and sensitive technologies has enabled the identification of potential biomarkers even when present in small quantities, thereby signifying the use of saliva as an important assessment media [2].

Salivary diagnostics is an important and significant development in disease diagnosis and treatment delivery. The need for diagnosis of oral and systemic disease and monitoring health regularly is the need of the hour, and saliva owing to its noninvasive and ease of collection along with the ability to detect a myriad of biomarkers is a promising tool. Salivary analytes have been evaluated for their role as potential biomarker in dental caries, periodontal diseases, oral microbial infections such as HIV, autoimmune disorders such as Sjogren's syndrome, drug and hormone monitoring, as well as in diagnosis of systemic diseases. Additionally, salivary diagnostics is emerging as an important investigation in the identification of tumor-specific biomarkers for early detection, diagnosis, prognosis, and therapeutic interventions of oral and oropharyngeal cancers as well as in cancer of distant sites like breast, prostate, cervical, and ovarian cancer. Research is being increasingly undertaken to identify saliva-based biomarkers which either singly or as a panel could be of significant use. Once these biomarkers are quantified and validated, they can be of use in point-of-care diagnostics for chairside detection of various human diseases.

A thorough knowledge of the functions and properties of saliva, its role in health and disease, and the diagnostic technologies available for the identification of saliva biomarkers is of paramount importance to further the knowledge on their usefulness in clinical research and patient management. This formed the basis of the current book on saliva which could serve as an important guide for future research involving salivary diagnostics.

The scientific literature on using saliva as a diagnostic tool began to emerge since the 1960s. Earlier attempts on the use of salivary diagnostics were confusing owing to the lack of uniformity in the methodology of salivary analysis and the use of available instrumentation. Over a period, the ability to determine salivary components has achieved a sense of uniformity and precision which could be attributed to the understanding of methods of handling saliva samples in various investigating laboratories. The chapters of this book attempt to highlight the advancements of salivary diagnostics over the years, the identification of various salivary-based biomarkers in oral and systemic diseases as evident in the literature, and the future considerations in employing saliva as a diagnostic tool.

The book intends to provide an overview of salivary constituents; the functions of saliva; the role of salivary diagnostics in various oral diseases, oral cancer, and breast cancer; and the determination of salivary DNA for forensic analysis.

## **2. Saliva in health**

Saliva is a complex fluid composed of 99.5% water and 0.5% solid material which is inclusive of organic and inorganic constituents. Whole saliva is composed of secretions from the major and minor salivary glands, gingival crevicular fluid, oral mucosal transudate, nasal and pharyngeal secretions, and desquamated epithelial cells. The functions of saliva include but not limited to lubrication, digestion, antimicrobial activity, buffering, and remineralization of tooth enamel. These functions are performed by the different components of saliva [3]. Numerous organic compounds such as proteins, antimicrobials, immunoglobulins, and blood group substances and various inorganic components are present in varying quantities in saliva [4]. The components of saliva are either an inherent component of saliva itself or are derived from blood. The component of plasma may enter into saliva through various processes like ultrafiltration through gap junctions, between cells of secretory units, transudation of plasma compounds into oral cavity through crevicular fluid, and by passive diffusion of lipophilic molecules [2]. The source of information in saliva is largely derived from the variety of DNA, RNA, and proteins present in saliva. Salivary DNA represents the genetic information of the hosting human body, the oral microbiota, and infecting DNA viruses. Salivary RNA provides information on transcription rates of the host genes as well as oral microbiota. Salivary proteins represent genetic information and help understand the translational regulation of the host body [5]. Cell-free saliva has been found to contain over 1000 proteins involved in a wide range of biological functions, mRNA and microRNA transcripts, and metabolites [6].

Specimen collection is an integral part of clinical research for identification and characterization of tumor-related biomarkers. Saliva can be collected under both resting and stimulated conditions. The duration of collecting period is important because flow rates vary with time. It is hence important to follow a standardized collection procedure during the whole sampling period to keep the secretion rate as constant as possible [7]. Further to collection of saliva, it is necessary for appropriate storage of the sample before analysis. The collected saliva should be kept on ice, aliquoted, and frozen as soon as possible to maintain the sample integrity [8]. Certain approaches to store saliva to prevent degradation of salivary compounds include immediate storage without any processing; if analysis is to be done within 30–90 min, saliva can be stored at room temperature; for analysis after 3–6 h after collection, storage is to be done at +4°C; and if analysis is done after days to months after collection, storage is to be done at –20°C or still better at –80°C [2].

The diagnostic techniques employed in the analysis of the processed saliva have made exponential progress over time. Previous techniques for analysis included calorimetric analysis, spectrophotometry, and few optical techniques. These methods have proven efficacy in serum/plasma analysis but did not find similar success in saliva. This could be attributed to the decreased concentration of the various analytes in saliva which demanded a more sensitive and specific technique to enable their identification. The application of liquid chromatography, gas chromatography, flow cytometry, and ELISA was successful in this regard. More recently several techniques such as nuclear magnetic spectroscopy and liquid chromatography coupled with mass spectrometry have been highly successful. Nuclear magnetic resonance spectroscopy enables the identification and simultaneous quantification

of a wide range of organic compounds in micromolar range [9]. The advantages of NMR spectroscopy are its high resolution, minimal sample preparation, and time-saving procedure [10]. Mass spectrometry is a method used to analyze samples either directly or following chromatographic or electrophoretic separation [11]. The coupling of liquid chromatography with spectrometry has enabled the identification of low-abundance salivary metabolites with very high sensitivity [12].

### 3. Saliva as a diagnostic tool

The complex nature of saliva consisting of mixture of components has posed difficulties in identification of specific constituents. However, recent inroads made in the discovery of highly sensitive technologies have enabled the identification of salivary biomarkers with high precision even when present in minute quantities. The role of saliva as a diagnostic tool was evaluated in HIV, oral cancer, as well as in cancer of other sites to name a few.

The need for chairside diagnosis and personalized medicine has led to an increase in the use of fluid-based biomarkers in disease diagnosis and treatment planning. Research involved in identification of disease-specific biomarkers in the blood, urine, and other body fluids was not forthcoming. The lack of absolute success of serum biomarkers along with the improvement in saliva collection and testing presents a unique opportunity for salivary diagnostics.

Molecular alterations are usually attributed to the inherent biological properties of human cell which may contribute to the disease progression. Identification of these alterations can assist in early detection and prediction of prognosis of various diseases including cancer. A comprehensive approach is based on a detection of panel of molecular alteration which can serve as an ideal biomarker for specific diseases. The rapid advancements in the identification of these molecular targets have something to do with the evolution of “omics” group of diagnostic aids [13]. Omics are scientific disciplines constituting the study of related sets of biological molecules. These include genomics, epigenomics, proteomics, transcriptomics, and metabolomics. The term “salivaomics” was coined in 2008 to reflect the rapid development of knowledge about the various omics constituents of saliva [14]. The substantiation of salivaomics technology has led to the discovery, validation, and application of salivary biomarkers in early diagnosis, treatment planning, and prediction of prognosis. Additionally, the development of bioinformatics and statistical tools aids in determining the discriminatory biomarkers in saliva for various oral and systemic diseases [14].

### 4. Salivaomics

Salivary diagnostics are being extensively reviewed to identify potential biomarkers using these omics technologies [6]. Ever since its discovery, metabolomic investigations can generate quantitative data for metabolites which offers a promising clinical strategy to characterize the association between salivary analytes and a disease [1].

Genomics defines the genetic message and the resulting protein sequences [15]. Genomic analysis in saliva includes the identification of disease-specific characteristics such as somatic mutations in tumor suppressor genes or oncogenes, microsatellite alterations, abnormal protein methylation, and mitochondrial DNA alteration [16]. The proteome represents the complete set of proteins encoded by the genome. Proteomics is the study of proteome that identifies the cellular levels

of all the isoforms and posttranslational modifications of the various proteins that are encoded by the genome of the cell under a given set of circumstances [5]. Salivary transcriptomics is defined as the study of the expressed mRNA transcript complement of a cell under different conditions. The large panel of human mRNA is determined using microarray technology followed by validation [17].

Metabolomics is one of the core disciplines of systems biology focusing on the study of low molecular weight organic and inorganic chemicals in biological system [18]. Metabolomics describes the full repertoire of small molecules present in cells, tissues, organs, and biological fluids [6]. The metabolomic profiles can be altered by various pathological processes, and global changes in these profiles may indicate the presence of a disease.

These form the diagnostic alphabets of saliva, and their discovery and quantification have resulted in the application of salivary diagnostics in clinical practice, in point-of-care diagnostics, as well as in personalized medicine.

## 5. Conclusion

Salivary biomarkers represent an accurate, noninvasive approach for detection of oral and systemic diseases and an attractive area of research. The development of robust and sensitive techniques for detection of salivary biomarkers, its quantification, and validation further maturing to clinical use is the need of the hour. There has been an upsurge in the laboratory-based tests for detection of salivary biomarkers which are ready for definitive validation through the use of various prospective clinical study designs. Researchers can now attempt to develop salivary biomarkers for early detection of diseases employing the omics constituents, saliva-based bioinformatics, and statistical tools. It is worth noting that the technology available enables one to detect promising and new salivary biomarkers as stand-alone tools without relying on their initial identification in the blood and other bodily fluids.

## Conflict of interest

The author does not have any conflict of interest to disclose.


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