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Saliva as a Diagnostic Tool in Dentistry – A Review

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Abstract

The identification of disease/pathogen plays a key role in diagnosis and treatment of a condition in particular. The disease causing pathogens such as bacteria, fungi and viruses are directly detected by various methods such as Polymerase Chain Reaction, Fluorescence in-situ Hybridization, Enzyme-Linked Immunosorbent Assay, Immunofluorescence, Flow cytometry. There are several types of specimens recommended for diagnosis of diseases including: serum samples, virology swab samples, biopsy and necropsy tissue, cerebrospinal fluid, whole blood for PCR, Saliva, urine samples and stool samples. The use of saliva to identify individuals with disease and to follow the progress of the affected individual has attracted the attention of numerous investigators. Its noninvasive method of collection, simplicity, and cost effectiveness make it a useful tool. The range of applications in salivary diagnostics is wide

from detecting caries risk to complicated newly emerging pandemic –Covid-19.

Introduction

Screening for disease prevention; Diagnosis at an early disease stage, when symptoms are just beginning to appear; Diagnosis and patient prognosis, particularly of infectious diseases to identify the causative agent and determine its resistance profile to antibiotics; Treatment decisions and treatment monitoring are vital steps irrespective of the disease. Diagnosis can be done in invasive and non-invasive methods. Salivary samples being a non-invasive procedure shares the following advantages; little or no pain, shorter recovery time, reduced risk of side effects and lower cost. It has become increasingly clear that the oral cavity can act as the site of origin for dissemination of pathogenic organisms to distant body sites, especially in immunocompromised hosts such as patients suffering from malignancies, diabetes, or rheumatoid arthritis or having corticosteroid or other immunosuppressive treatment.

From the adventurer's past, saliva diagnostics emerges into the clock-maker's present. Now it is time for us to exchange adventurer's leather jacket for a clock-maker's loupe. We are in the critical phase where the scientific foundations of saliva for clinical disease detection must be established beyond any doubt. Only on broadest foundation of absolute scientific integrity can be build the highest edifice of saliva diagnostics in the shortest possible time. Salivary diagnostic applications fall into several broad categories: Infectious agents, local and systemic diseases, hormone levels, therapeutic drug monitoring, drugs of abuse, forensic applications.

Many bio markers may be measured using oral fluids. This opens up the extraordinary opportunity if enhancing research conducted in the field or expanding the versatility of point of care diagnostics by using saliva as the diagnostic fluid. Behavioral research that seeks to correlate physiological with psychological status has been revolutionized by availability of assays that accurately measure such hormones as cortisol in saliva. Continued advances in biomedical engineering coupled to enhance understanding of salivary proteome will make it possible to one day implant biosensors directly in the mouth. These in dwelling "labs on a chip" will help catalyze a shift from our current practice of disease detection to a health surveillance through detection and measurement of multiple, relevant bio-markers in saliva.

With the advances of this technology comes the additional significant obligation to ensure the privacy of patients. While saliva collection is facile and anatomically non-invasive, oral fluids have sufficient quantities of DNA to decode genotypes. Thus, one must be equally discrete with saliva and blood samples.

Human saliva contains a repertoire of proteins, glycoproteins, lipids, metabolites, RNA, and genomic information, in addition to 700 microbial species and is endowed with many of the same diagnostic analytes inherent in other bodily fluids such as blood, CSF and urine. The National Institute of Dental and Craniofacial research charged into the inclement waters of risky science and planted its flag on the "terra incognita of saliva omics." The completion of human salivary proteome project is a land mark accomplishment marking the discovery of first diagnostic alphabet in saliva.

And the future? With the potential to revolutionize delivery of diagnostic services both on the personal and public health levels, the future of saliva diagnostics is enormous.

Mass spectrometry analysis has recently revealed that in addition to dozens of major secretory proteins, more than a thousand less abundant proteins are also found in saliva. However, hundreds of protein found in saliva are also present in blood plasma suggesting that plasma proteins make their way in to saliva. The blood plasma proteins released in to saliva are not likely o be secreted by granules. Because this latter study collected ductal saliva from individual glands of healthy individuals, the detection of plasma proteins in saliva does not represent contamination by GCF, food debris or bacteria. consequently, because the salivary gland acinar complexes are" leaky" epithelia, many of these proteins must enter saliva by diffusion across tight junction complexes. Regardless of mechanism of secretion of plasma proteins in ductal saliva, the results of this study suggest that saliva is an easily accessible source of monitoring numerous proteins that originate in blood[1].Thus in some cases salivary proteins serve as biomarkers.

Amongst all the functions of saliva (protection of oral cavity and oral environment, digestion, mastication, deglutition, taste perception, speech, tissue repair and excretion) the utmost important function is it can be used for diagnosis. Saliva and diagnosis are interrelated as proper and accurate diagnosis leads a straight route to hit the target (i.e disease).

Collection of saliva

Collection of whole saliva is gathering fluids that will flow outside the mouth which is not the entire amount of saliva, since part of it will remain coating oral surfaces. It should be collected always same time of day between 9 am to 11 am. Steps of Collection: Refrain from eating and drinking 90 min before collection stop the use of drugs that might affect salivary secretion for at least one day. Rinse mouth with de ionized water prior to collection .collect saliva for 10 min. various methods are draining, spitting, suction, absorbent method. Collection of saliva on filter paper can be a viable option[2]. Several saliva collection kits are already marketed and some have gained FDA approval for various diagnostic tests.

Processing and storage of saliva

Chemical stability of the bio marker as well as diagnostic purpose to a complete extent determines the way sample collected should be processed and stored. For example, collection, processing and storage of samples for analysis of ions such as sodium and potassium is less critical than for analysis of a highly labile enzyme. Standard collection of whole or glandular saliva in ice-coded vials. Vortexing (2 minutes, maximum speed), centrifugation are not needed for glandular saliva directly collected from orifice of particular gland using cannulation or a lashley cup. Snap freezing of whole or glandular saliva sample in liquid nitrogen. Storage at -20^{o}c or below, but preferentially at -80^{o}c.

Salivary Diagnostics: An alternative to more expensive, invasive testing procedures

Blood is a complex bodily fluid known to contain a wide range of molecular components, including enzymes, hormones, antibodies, and growth factors like saliva. Blood serum or plasma is traditionally and most frequently the source of measurable biomarkers, while cells, tissues, stool, and other alternatives are routinely pursued. Although life-saving in many instances, the procedures required to collect and eventually analyze blood samples can often be problematic, expensive, and physically intrusive. Employing salivary fluids as a medium for biomarker development and evaluation alleviates subject/patient discomfort through the provision of a noninvasive method of disease detection.[3]

Application of Mass Spectrometry (MS) to the saliva field

The application of Mass Spectrometry (MS) technology to whole saliva and glandular secretions led to new insights of their characteristics and identification of proteins that were not known at the time to be part of this body fluid. Significant interest in developing saliva-based bio markers for diagnostics has fueled the establishment of various saliva proteome projects.

Role of microfiber array technology in salivary diagnostics

Quite distinct from the MS analysis, the latter technologies utilize chromophoric fluorescent and immune reactivity detection systems[4]. Optical fiber array methodology is uniquely suited for application in a multivariate model. High-density arrays for multianalyte sensing provide a tool not only to identify but also to quantify simultaneously multiple specific biomarkes at the femto- to nanomole level. This technology has also proved to be amenable to arrays with sensors reacting with more than one analyte, each triggering different signal outputs.

Role of salivary biomarkers

Biomarkers present in saliva are being currently analyzed or investigated as potential tests for a multitude of clinical tests. Various biomarkers of saliva; viral DNA/antibodies, bacterial DNA and antibodies, steroid hormones, drugs and c-erbB-2 protein are associated with diagnosis of caries susceptibility[5]..Salivary levels of antidengue IgM and IgG can be used to distinguish between primary and secondary infections [6,7]. In oral squamous cell carcinoma (OSCC) patients, combined testing of salivary levels of Cyfra 21-1, TPA, and CA125 had a similar diagnostic value as measuring the same markers in patients sera. Testing of saliva for antibodies to human immunodeficiency virus (HIV) has been practiced, and the assay is equivalent to serum in its accuracy with the advantage of being safer and easier to use[8].The presence of a wide array of biological markers such as proteins, glycoproteins, DNA, RNA, small molecule metabolites and messengers, drug metabolic byproducts, bacteria, viruses, exfoliated cells, and antibodies highlight the promise that saliva diagnostics offers the future of clinical testing.

Salivary Transcriptome

The presence of cell-free human transcripts in saliva opens up new possibilities of non invasive gene expression profiling. Using the U133A high-density oligonucleotide microarray from Affymetrix global profiling of the salivary transcriptome led to the generation of a reference database of over 3,000 RNA species[9].A study was conducted to explore the transitional utility of the salivary transcriptome: Saliva mRNA from patients with primary T1/T2 oral squamous cell carcinoma was compared to healthy subjects' profiles[10].Real-time PCR validation confirmed seven mRNAs that were at least 3.5-fold elevated in saliva from cancer patients. These salivary RNA biomarkers are transcripts of DUSP1, HA3, OAZ1, S100P, SAT, IL8, IL1B.With the salivary proteome and transcriptome diagnostics alphabets, information related to oral and systemic diseases that come in many forms can be envisioned to be harnessed from a single drop of saliva.

Saliva in dental caries risk assessment

A model for the caries susceptibility test is proposed that attempts to incorporate fundamental features of caries test into a context of functional elements of saliva. This model suggests that the oligosaccharide group is positively correlated with caries history associated primarily with glycoproteins that contribute to pellicle formation. Alternatively, oligosaccharides that are negatively correlated with caries history would be associated with salivary glycoproteins that function to agglutinate oral bacteria. The ratio of these two groups of oligosaccharides provides the best correlation with caries history and is the best predictor of risk of future caries in children[11].

Saliva in Periodontal disease

Proteins and peptides secreted from major salivary glands are responsible for maintenance of oral cavity integrity. Because of its importance in oral biofilm formation and host defense, secreted saliva has a significant role in the establishment and progression of periodontal disease. Peroxidase is a salivary enzyme produced by acinar cells in salivary glands. It removes the toxic hydrogen peroxide produced by oral microorganisms and reduces acid production in dental biofilm, decreasing plaque accumulation and establishment of gingivitis and caries. Patients with periodontal disease have demonstrated high levels of this enzyme in saliva[12]. Saliva is used as important screening test for osteoporosis. CRP has recently been measurable in saliva from periodontal patients using a "lab-on-a-chip" method[13].

Saliva in Forensic dentistry

The presence of a bitemark means that the mouth of the offender has made contact with an object. Such contact will almost certainly leave some trace of saliva. This can be an important source of DNA that can be used for identification purposes. Saliva contains skin cells from the lining of the oral cavity. These cells each contain a nucleus that possesses a nuclear DNA. The concentration of these cells is quite high in human saliva and allows recovery of potentially identifying information on whom or what made the bite mark. The presence of a Y chromosome in the resulting profile indicates that the male was the biter. The lack of a Y chromosome and the presence of X chromosome mean that a female was the biter.

Saliva in COVID-19

Saliva is a biological fluid in which SARS-CoV-2 can be found and for this reason saliva has been taken into consideration in the diagnosis of COVID-19.[14] SARS-CoV has been shown to infect epithelial cells in salivary gland ducts in rhesus macaques.[15] The presence of SARS-CoV-2 in saliva may be related to different sources such as i) virus entry to the oral cavity from lower/upper respiratory tract, ii) access to the mouth via oral cavity-specific crevicular fluid or iii) release of viral particles in the oral cavity via salivary ducts from the infected salivary glands. The potential use of saliva for SARS-CoV-2 detection is scientifically well founded.

Saliva is considered to be a good reservoir for viruses that originate from oral shedding, and secretions from the lower respiratory tract, naso-pharynx and possibly infected salivary glands. When coughing, speaking, sneezing, or even breathing, saliva droplets are produced and shaped as particles in a combination of droplet nuclei of microorganisms and moisture. Each cough can produce about 3000 saliva droplets nuclei, which is approximately equivalent to the quantity generated during a 5-min talk. Each sneeze can produce roughly 40,000 droplets of saliva covering several meters in the air. A regular exhalation may create saliva droplets that exceed one meter in the air. Huge saliva droplets with more mass typically fall to the ground and small saliva droplets fly by airflow over longer distances.[16] Chen *et al* were able to detect SARS-CoV-2 RNA in three out of four saliva samples directly collected from the salivary gland ducts, thereby precluding contamination from respiratory secretions, of critically ill cases.[17]The search for salivary biomarkers associated with the development and progression of COVID-19 could allow a better distinction between asymptomatic, mild, moderate or advanced disease.

Barriers to development and widespread use of salivary diagnostics

According to the National Institute of Dental and Craniofacial Research (NIDCR) work shop-1999 on development of new technologies for saliva and other oral fluid-based diagnostics, it was noted that three general types of barriers to wide-scale implementation of salivary diagnostics.

The need for additional basic and applied research-for example, the need to design and develop micro-sensors capable of accurate measurements in small volumes and standardization of collection methods

-The need for product development; and

-Third-party acceptance and associated legal issues.

Future Advances

A test that would enable patients at risk of a fatal disease to be screened frequently and easily would not only save lives but also have a dramatic effect on morbidity. Recent scientific advances and the priority that is currently being given to research in saliva diagnostics mean that this area of research will continue to grow.

Conclusion

Saliva offers an alternative to more invasive, time-consuming, complicated, and expensive diagnostic approaches. Effective use of salivary diagnostics requires a willingness by patients to share salivary diagnostic test results with their physicians. Salivary diagnostics may yet achieve a key role in routine health monitoring. This enables the early diagnosis of oral and systemic disease at a stage when intervention can be much simpler and more effective.

Therefore, salivary diagnostics will not only help save lives but also perverse the quality of those lives that have been saved.

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