RECENT ADVANCES IN SALIVARY DIAGNOSTICS

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Abstract:

Saliva has been the most efficient diagnostic tool since ages, its progress from being a lie detector in the times of the ancient Greeks to the present evolution into a master tool in the much talked about metabonomics. This review article attempts to show the way how saliva has reached its potential to serve the status of a potential diagnostic aid. This article also focuses on the developments made in the past and also the futuristic plans in the field of salivary diagnostics.

Keywords: saliva, diagnostic aids, desquamated epithelium

Introduction

Blood has been the most commonly used laboratory diagnostic procedures which involve the analysis of its various constituents of blood. Among other biologic fluids, saliva offers distinct advantages, saliva can be collected non invasively and by individuals with limited training, costs towards the procedure are dramatically reduced as no special equipments are required for the collection of saliva. Saliva is a mixture of various secretions from major and minor salivary glands, including even those which are non salivary in such as gingival crevicular fluid, bronchial and nasal secretion, serum and blood derivatives from oral wounds, bacterial products, viruses, fungi, desquamated epithelial cells, other cellular components and food debris[1,2,3]. As, serum can also reach the saliva through other means such as ultrafiltration, passive diffusion (most common) and active transport, both gland specific and systemic pathologies can be detected by saliva analysis [4,5,6]. The purpose of this article is to review on the recent advanced diagnostic applications of saliva.

To achieve the above goal i.e. to prove as an efficient diagnostic tool, different biomarkers for various diseases need to be identified from the routine composition of saliva. For this, the tools used should have a prerequisite of advanced sensitivity and specificity of the biomarker targeted.

Discussion

In the past few decades, scientists have demonstrated abnormal nucleic acids and/ or proteins from the saliva using techniques like Enzyme Linked Immunosorbent Assay, Western Blot, Conventional/ Qualitative Polymerase Chain Reaction(Fig. 1).

Besides creating a significant impact on research, molecular diagnostics have proved to be efficient in clinical applications[9,10] In the past few decades diagnosis of diseases by using saliva as the main tool remained impossible, hence clinical features also had to be taken into consideration. Systemic diseases like cancer, cardiovascular, neurological and metabolic diseases. Large number of diagnostic analytes have been shown and discovered in saliva, including steroid hormones, HIV antibody and infections.

Oral health researchers have also been trying to develop diagnostic tools to monitor periodontal diseases and caries risk assessment[5,6,7]. One of the aims researchers are pursuing is a comprehensive bench-to-bedside program to understand the molecular basis of the human body function and to develop strategies for their diagnosis as well as prevention, and management.

Recent discoveries and progress

Investigators are trying to catalogue the human saliva proteome, to identify salivary biomarkers for human diseases, and also compile a list of proteins expressed in salivary glands. Wong et al in 2008 collected, catalogued and analyzed human saliva. Out of 1166 identifications, 914 were found in parotid and 917 in submandibular/sublingual were made. This showed that a significant portion of the proteins found in plasma was also found in saliva[11]. Work on identification

and analysis of altered gene expression lead to the discovery of four messenger RNA (mRNA) biomarkers -- KRAS, MBD3L2, ACRV1 and DPM. These helped to differentiate pancreatic cancer patients from non-cancer subjects[12]. Researchers collected saliva samples from 64 patients with oral squamous cell carcinoma and 64 healthy patients. Five candidate biomarkers were successfully validated using immunoassays: M2BP, MRP14, CD59, profilin and catalase. The presence of these biomarkers confirmed the presence of oral cancer[13]. This comparison has provided a scientific foundation to separate potential protein markers of the disease[14].

In a study mean levels of IL-1 and MMP-8 in saliva were significantly higher in subjects with periodontal disease than in controls. The biomarkers correlated with individual clinical parameters indicative of periodontal disease[16].

In an era where diabetes is a global scare potential biomarkers were identified and characterized to detect prediabetes. This was designed to detect or prevent progression to frank diabetes and its complications. Multidiemensional liquid chromatography/ tandem mass spectrometry was used on whole saliva and 487 unique proteins were identified, 33% of them not identified before in human saliva. This was the first global view of potential mechanisms and their utility in detection and monitoring of diabetes[17].

Potential diagnostic technologies tools of the future

Metabonomics: is "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications". This analytical platform has the concept of applying of NMR and MS spectroscopies to study the metabolic composition of biological fluids, cells, and tissues[15].

In the recent past research initiated by the NIDCR (National Institute of Dental and Craniofacial Research) research efforts in the area of salivary diagnostics have made significant research. Microfluidics and Microelectromechanical systems (MEMS) being a few potential futuristic tools. MEMS are the latest and are composed of integrated systems with mechanical elements, sensors, actuators and electronics on a common silicon substrate developed through microfabrication technology. These systems use small samples and reagent volumes with integrated detection methods to perform an analysis. This technology can be used for measuring proteins, DNA, gene transcripts (mRNA), electrolytes and small molecules in saliva.

Disease	Defect	Saliva	Reference
Systemic diseases			
Cystic fibrosis	Mutations	Increased electrolytes (esp calcium) Unusual EGF form Increased prostaglandins (PGE2)	Mandel et al 1967 Hubert et al 1990 Rigas et al 1981
21-Hydroxylase deficiency	Deficiency of 21 hydroxylase leads to congenital adrenal hyperplasia	Early morning levels of 17 hydroxy progesterone	Zerah et al 1987
Autoimmune diseases			
Sjogrens Syndrome	Unknown etiology	Low resting and stimulated salivary flow rate (0.1ml/min resting, 0.5ml/min stimulated) Elevated concentration of sodium and chloride Elevated levels of IgA, IgG, lactoferrin, albumin, decreased concentration of phosphate Elevated β2 microglobulin SS anti La antibodies.	Sreebny et al 1996 Tishler et al 1997 Ben Aryeh et al 1981 Michaelski et al 1973
Malignancy			
	Gene deletions and mutations	P53 antibody[17] Increased salivary defensins[18]. Elevated levels of tumor markers (c-erbB2 & CA 15-3)[19] Salivary nitrate and nitrosamines	Tavassoli et al 1998 Lichtenstein et al 1986 Streckfus et al 2000 Tenovuo et al 1986
Infectious diseases			
Helicobacter pylori		Production of specific IgG antibody. H pylori DNA	Is et al 1996
Shigellosis	Shigella	Antilipopolysaccharide, anti shiga toxin[20].	Schulin et al 1992
Pigeon breeders disease		Pneumococcal C polysaccharide[21].	Kazek et al 1986
Lyme disease	Borrelia burgodorferi	Antitick antibody[22]	Schwartz et al 1991
Viral			
Hepatitis	Hepatitis A virus	Levels of IgM antibody	Bull et al 1999, Smart et al 1992
	Hepatitis B virus	Hepatitis B surface antigen	Chiou et al 1993
Rotavirus		Salivary IgA response	Jayashree et al 1988
Herpes virus	Epstein Barr virus, Cytomegalovirus, Herpes simplex virus	Shedding of viruses in saliva	Blackbourn et al 1998
Human Immunodeficiency Virus		Salivary IgA levels prognostic marker	Matsuda et al 1993
Drug monitoring			
Marijuana			Gross et al 1985
Tobacco smoking		Cotinine, Salivary thiocyanate	Benowitz et al 1983 Luepker et al 1981

Fig. 1

Conclusion

The developments made in the area of diagnostic salivary biomarkers such as proteomic and genomic technological developments would lead to the development

of extremely efficient tools for making important clinical decisions and also to predict treatment outcomes. Barriers such as achieving high sensitivity, high specificity, procuring results for a large number of samples simultaneously, have been overcome and come a long way in assisting clinical diagnosis. These developments are of particular importance in developing countries where access and affordability plays a critical role.

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