SALIVARY DIAGNOSTICS – RELOADED

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Abstract

As we approach the threshold of genomic medicine, the increasing use of salivary diagnostics will help catalyze a shift from disease diagnosis to health surveillance. With new techniques for detecting small quantities of salivary components, including proteins and messenger RNA (mRNA), the field of salivary diagnostics has emerged as one of dentistry's most promising areas of research. Because collecting saliva is noninvasive, it is becoming the preferable way in bridging state-of-the-art saliva-based biosensors and disease-discriminatory salivary biomarkers in diagnostic applications.

The challenge to make salivary diagnostics a clinical reality is in establishing the scientific foundation and clinical validations necessary to position it as a highly accurate and feasible technology, which can achieve definite point-of-care assessment of patient health and disease status.The field of salivary diagnostics is now becoming a broad, complex and crosscutting area of scientific research with enormous potential to impact the practicing dentist and health care in general.

Introduction

Since time immemorial, saliva has been portrayed as a unique yet complex body fluid. We are keenly aware that adequate saliva is essential in maintaining oral health. As a commonly easily accessible oral fluid, saliva plays a central role in several legends and anecdotes. While spitting is something that is viewed with scorn, saliva can also appear quite charming when falling from a baby's mouth.

Though saliva(spit) is usually portrayed in negative light in the eyes of the lay population, a range of interdisciplinary research is well underway that may rapidly change the negative perceptions about saliva in a few years.

Early milestones in salivary diagnostics

The ancient Greeks were among the first to recognize the medicinal value of saliva over 2000 years ago when they placed non-venomous snake saliva on wounds to aid in healing.(**1**)Studies in saliva were already being done in around the 1890's, when Chittenden et al conducted the study of the influence of alcoholic drinks upon digestion and secretion where the measurement of total organic constituents, salts and chlorine in saliva was assessed.(**2**) .

The earliest "sialochemical" studies on oral fluids were conducted by Michaels and Kirk in the 1900's,each of whom examined saliva for specific

components that would be diagnostic for various systemic conditions, including gout and rheumatism. As researchers started to learn more of these properties,unstimulated whole saliva and gland specific saliva
gradually became the samples of common choices,and standard collection techniques were established to minimize physiological variations **(3).**

Need for salivary diagnostics

Oral fluid/Saliva, called the 'mirror of the body', is a perfect medium to be explored for health and disease surveillance. While it is relatively inexpensive,the non-invasive, simple and accurate screening methods provide obvious patient benefits that consequently creates an ideal opportunity to bridge the state-of-the-art saliva-based biosensors and disease-discriminatory salivary biomarkers in diagnostic applications.

In 2002,The National Institute of Dental and Craniofacial Research(NIDCR)-USA, initiated a concerted research effort in the area of saliva diagnostics through the funding of microfluidics and microelectromechanical systems (MEMS) for saliva diagnostics. MEMS are integrated systems consisting of sensors, actuators and electronics on a common silicon substrate developed through microfabrication technology. In 2003, the NIDCR made the serendipitous discovery that discriminatory and diagnostic human mRNAs are present in saliva of normal and diseased individuals. **(4)**
3000 mRNAs were discovered in the normal salivary transcriptome.Of these, 180 are common between different normal subjects, constituting the normal salivary transcriptome core (NSTC). **(5)**
Empowered with new and highly sensitive technologies, the lower level of analytes in saliva is no longer a limitation. Almost anything that can be measured in blood can also be measured in saliva.(Fig.1)

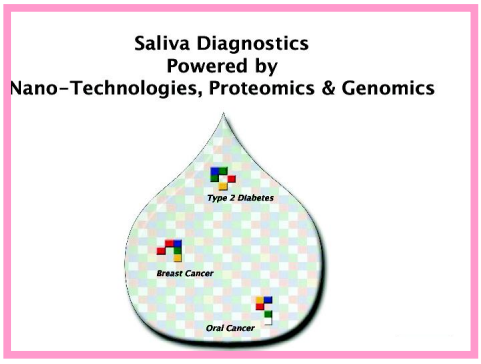

Fig1.depicts the role of Saliva Diagnostics in systemic diseases

Clinical applications

With the establishment of the saliva research base,dental and biomedical researchers now have the scientific foundation upon which it can develop clinical tools that accurately screen salivary samples for the presence or absence of disease-specific biomarkers.(Fig.2)

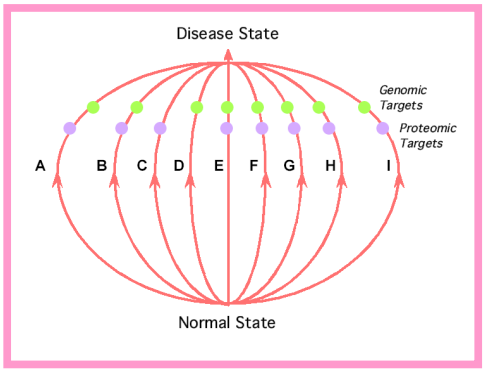

Fig.2 depicts the Saliva Research toolbox of specific molecular markers

<u>Detection and Measurement of Drugs</u>

It is used to monitor a variety of drugs including marijuana,cocaine and alcohol.

(ORALERT- ORAL SALIVA DRUG DETECTION KIT)

Detection and Measurement of Hormones

A trial of 956 women found that a single positive salivary estriol test predicted an increased risk of spontaneous preterm labor and delivery in both asymptomatic and symptomatic as well.**(6)** Recent studies have also demonstrated that night time salivary cortisol sampling is a facile approach to screening for Cushing's syndrome in children.(**7)**

The neuroendocrine profiles ,obtained from using saliva samples of subjects experiencing military survival training, showed increased *cortisol levels* during their captivity experience, and peaked following routine interrogation.

Detection of Systemic Diseases by using Saliva

Cardiovascular Diseases

Salivary endothelin concentrations are elevated in persons with chronic heart failure, and these levels can be used to assess disease severity. **(8)**
Salivary TC, TG, LDL-C and VLDL-C concentrations were significantly high in patients with ischemic stroke when compared with risk and control groups in a study of 150 individuals as reported by Natheer AL-Rawi from the Baghdad University.

In 2009,the San Antonio Emergency Medical Team(EMT) crews in the USA, began using a spit test that detects cardiac arrest faster, more accurately and more cheaply than other diagnostic tests. Engineered by researchers at the University of Texas, the chip can measure proteins in saliva that signal heart attacks long before the ambulance pulls into the ER. (tastechip.com)

Viral Infections

Saliva has been reliably used to detect HIV-1 and 2, and viral hepatitis A, B and C.

Early Diabetes

This proteomic analysis of the human saliva in type 2 diabetes provides the first global view of potential mechanisms altered in diabetic saliva and their utility in detection and monitoring of diabetes. These may provide the basis for new, non-invasive tests for diabetes screening, detection, and monitoring.

Periodontal Diseases

Another oral disease where salivary diagnostic technology is showing promise is the detection and monitoring of periodontal disease. This application includes the assessment of molecules related to the host inflammatory response and the destruction of associated connective tissue, as well as detection of specific pathogenic bacteria and bacterial products.

Detection of Oral Cancer

Based on levels of specific molecular markers,molecular staging profiles have been identified in saliva as *objective prognostic indicators.*Researchers have found that panels of salivary mRNA (messenger ribonucleic acid) and protein markers can serve as biomarkers for oral cancer detection.
Scientists confirmed the validation of five biomarkers namely M2BP, MRP14, CD59, profilin and catalase. The presence of these biomarkers further assured the certainty of oral cancer nearly 93% of the time as each of them was also individually linked to oral cancer. Their research samples showed a 90% sensitivity and 83% specificity for oral squamous cell carcinoma.**(9)**
Another study conducted in Dharwad, India showed significant high levels of total protein and

sialic acid content in salivary samples of 30 OSCC patients. **(10)**

In a recent study,the salivary CEA and CA-50 levels were reported to be higher in salivary samples of 80 patients with malignant salivary gland tumors.**(11)**

Implications of Salivary Diagnostic Research for the Dental Profession

The role of rapid, in-office screening or chairside diagnostic testing comes down to an important bottom line: improved access and health care outcomes for patients. Saliva-based tests also offer the following advantages for patients and providers:

- Ease of collection
- Elimination of the common fear of needlesticks
- Lower costs for sample collection; and
- Reduced risks of percutaneous injury.

Salivary diagnostics could dramatically change the clinical practice by introducing point-of-care testing and real-time disease surveillance. Dentistry can help this vision become a reality, because many patients see their dentists more often than their physicians because of the concern for prevention focus and the need for regular visits to maintain oral health.In doing so, the dentist has an opportunity to be the primary health provider and, help aid in the early detection of disease and provide quality health care.

References:

1. Kongara KR, Soffer EE. Saliva and oesophageal protection. Am J Gastroenterol. 94(6):1446-52.

2. Chittenden, R. H., L. B. Mendel, et al.(1898). "A Further Study of the Influence of Alcohol and Alcoholic Drinks upon Digestion,with Special Reference to Secretion." American Journal of Physiology 1: 164 - 209.

3. Ferguson, D. B. (1987). "Current diagnostic uses of saliva." J Dent Res 66(2): 420-4.

4. Li Y., St John M. A., Zhou X., Kim Y., Sinha U., Jordan R. C., Eisele D. , Abemayor E., Elashoff D., Park N. H. and Wong D. T. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res.2004 ; 10 : 8442-8450.

5. Li Y, Zhou X, St John MA, Wong DT. RNA profiling of cell-free saliva using microarray technology. J Dent Res 2004: 83:199–203.

6. Heine RP, McGregor JA, Goodwin TM, Artal R, Hayashi RH, Robertson PA, Varner MW. Serial salivary estriol to detect an increased risk of preterm birth. Obstet Gynecol 2000;96:490-7.

7. Gafni RI, Papanicolaou DA, Nieman LK. Nighttime salivary cortisol measurement as a simple, noninvasive, outpatient screening test for Cushing's syndrome in children and adolescents. J Pediatrics 2000; 137:30-5.

8. Denver RA, Tzanidis A, Martin P, Krum H. Salivary endothelin concentrations in the assessment of chronic heart failure. The Lancet 2000;355:468-9.

9. Hu S, Arellano M, Boontheung P et al. Salivary proteomics for oral cancer biomarker discovery. Clin.Cancer Res 2008;14(19):6246-6252.

10. Sanjay PR et al. Evaluation of salivary sialic acid, total protein, and total sugar in oral cancer: A preliminary report. Indian J Dent Res. 2008; 19(4): 288-291

11. Hong He,Guanfu Chen,Liang Zhou,Yanmin Liu. A joint detection of CEA and CA-50 levels in saliva and serum of patients with tumors in oral region and salivary gland.
J Cancer Res Clin Oncol (2009) 135:1315–1321

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