Indian Journal of Dental Sciences.
December 2011 Issue:5, Vol.:3

www.ijds.in

Original Article

Indian Journal
of Dental Sciences

E ISSN NO. 2231-2293
P ISSN NO. 0976-4003

Salivary Diagnostics: An Insight

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Date of Submission : 26th September 2011
Date of Acceptance : 5th October 2011

Abstract
Saliva is a diagnostic medium that can be easily collected and with minimal invasion but it has been neglected in the past. It is now systematically being researched and oral fluid analysis is being compared with the analysis of other diagnostic media such as blood and urine. Today, saliva is being used more often to diagnose: hereditary diseases, autoimmune diseases, infectious diseases, endocrine disorders as, well as in the assessment of therapeutic levels of drugs and in forensic field. A number of recent studies have focused on oncogenic marker detection and its monitoring in saliva. The latest clinical and laboratory findings on diagnostic markers of oropharyngeal carcinoma in saliva could be the beginning of their wider use as a diagnostic medium. With the addition of modern techniques and chemical instrumentation equipment, there has been an increase in its use for laboratory investigations, applicable for basic and clinical analyses in the fields of medicine and dentistry. The value of these methods for the diagnosis of oral and systemic diseases has been the subject of study by several researchers with the aim of increasing its use alongside complementary exams.

Key Words
saliva, biomarkers, noninvasive, diagnostic fluid, systemic diseases

Introduction

"Saliva is a clear liquid secreted into the mouth by the salivary glands & mucous glands of the mouth."Saliva is one of the most complex, versatile, and important body fluids, supplying a large range of physiological needs.[1,2] The fluid has an old history of study but its physiological importance has only been recognized recently. In the past 50 years the pace of salivary research has accelerated with the advent of new techniques that illuminated the biochemical and physicochemical properties of saliva. Interest in saliva increased more with the finding that saliva is filled with hundreds of components that may serve to detect systemic disease or evidence of exposure to various harmful substances, as well as provide biomarkers of health and disease status.[3,4] The pithy expression by Mandel (1990) was that "saliva lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears".[5] Despite the absence of charisma, the diversity and the orchestration of the many protective agents in saliva started to become clearer during 1960s to '70s, when new molecules with multiple functions were repeatedly revealed and explored.[5] Although historically scorned in literature, viewed by many cultures as the ultimate insult and clinically "damned," investigators, clinicians, and our patients are increasingly turning to saliva as a safe and "non-invasive" indicator of health and disease. Many biomarkers may be measured using oral fluids. Saliva fulfills several of the chief diagnostic concerns for a diagnostic biofluid in as much as it is obtained noninvasively requiring no special skill. (ii) saliva flow rate and ionic composition are easy to measure by straightforward methods (iii) recent advances in understanding the salivation mechanism should help to confirm the molecular basis of many disorders and thus provide critical information for developing more specific and efficient treatment modalities. This opens up the extraordinary opportunity of enhancing research conducted in the field or expanding the versatility of point-of-care diagnostics by using saliva as the diagnostic fluid.[6] Saliva has been demonstrated to be an excellent test material for many types of systemic markers including those for endocrine function, stress or psychological state, exposure to infectious agents, use or metabolism of drugs or other xenobiotics, and other cancers.[6] This list of uses of saliva is increasing day by day as more and more molecules and markers have been described.[1]Nowadays, the saliva research field is rapidly advancing due to the use of novel approaches that include metabolomics, genomics, proteomics and bioinformatics. However, the use of saliva as research material may pose particular problems due to its complex biochemical and physical chemical properties and its inherent variability and instability.[4]

Methods Of Collection

Several methods have been suggested to collect resting and stimulated whole saliva. Most commonly, saliva is collected by draining or spitting into a tube, or chewing on an absorbent material. Collection of pure glandular secretions is possible with the use of special collection devices, for example the Lashley-cup for collection of parotid saliva. Standardization of saliva collections is important when saliva is used as research material, since saliva composition varies greatly both intra- and inter-individually. Non-standardized methodology has partly contributed to the high variability in the data published for saliva parameters, like composition, viscosity and lubrication properties.[4]

Saliva-Based Diagnostic Technologies

The barriers to widespread implementation of saliva diagnostics derive from

technological problems in achieving sensitivity, miniaturization, high throughput, automation, portability, low cost, high functionality, and speed to enable high-content chemical and biochemical analyses Today, the potential of microsensor and microfluidic technologies to facilitate the decentralization of medical testing is becoming accepted as one element in the next evolutionary stage of healthcare. Microfluidic systems can be designed to obtain and process measurements from small volumes of complex fluids, such as saliva, with efficiency and speed, and without the need for an expert operator. This unique set of capabilities is precisely what is needed to create portable point-of-care medical diagnostic systems allow miniaturization, integration, and multiplexing of complex functions,- could move diagnostic tools out of the laboratory and closer to the patient.[6]**Microsphere-based** nano-bio chip: Microfluidic lab-on-a-chip device is being designed and developed that mates with bead-based microarrays to detect cytokines/chemokines and nucleic acids in oral fluids. [6]**Microsphere-based optical fiber arrays:** Directed toward the development of a fully functional integrated platform that is capable of testing for both cellular and soluble analytes. Investigators are now focusing on the development of saliva-based cardiac diagnostic tests. [6]**On-chip PCR system, Electrochemical sensing system:** Intended clinical application of the OFNASET will be for the saliva-based molecular screening for oral cancer detection. Research group has identified five salivary proteins and seven salivary RNAs that are highly discriminatory for oral cancer.[6]

Salivary Biomarkers with Their Possibilities for Use

Saliva/Oral Fluid Biomarkers	Possibilities for use
DNA	Standard genotyping Bacterial infection Diagnosing carcinomas of head and neck Forensics
RNA	Viral/bacterial identification Carcinomas of the head and neck
Proteins	Diagnosing periodontitis Diagnosing carcinomas of the head and neck Detecting dental cavities
Immunoglobulins	Diagnosing viruses (HIV, hepatitis B and C)
Metabolites	Diagnosing periodontitis
Drugs and their metabolites	Monitoring drug abuse Detecting of drugs in the body
Viruses, bacteria	Epstein-Barr virus reactivation (mononucleosis)
Cellular material	Diagnosing carcinomas of the head and neck

Courtesy: Pink A et al . Saliva as a Diagnostic Medium. Biomed Pap Med Fac Univ (Palacky Olomouc Czech Repub. 2009, 153(2):103-110.)

The Diagnostic Potential Of Saliva

Blood will always be a primary bio fluid for diverse diagnostic tests, but it has distinct limitations and disadvantages. Other body fluids collected non invasively and without the need for skilled personnel can fulfill select niches for different diagnostic purposes. Saliva has shown its value for a number of tests already being widely implemented, but current research suggests the full potential of this bio fluid remains to be explored further. Saliva fulfills several of the chief diagnostic concerns for a diagnostic bio fluid in as much as it is obtained non invasively requiring no special skill. Several saliva collection kits are already marketed and some have gained FDA approval for various diagnostic tests.[4]

Advantages Of Saliva

Saliva collection is inexpensive. It is easy to collect, store, ship and it can be collected with the help of very little armamentarium and can be stored easily without requirement of any special instrument. Its collection is safer than blood tests, which could expose health care workers to HIV or hepatitis virus. It is easier to handle for diagnostic procedures, since it does not clot, thus lessening the manipulations required. Saliva based diagnostics is therefore more accessible, accurate, less expensive, and presents less risk to the patient than current methodologies.

Use Of Saliva As Diagnostic Fluid

Historically this diagnostic value may have been recognized first by the ancient judicial community who employed salivary flow (or its absence) as the basis for a primitive lie detector test. Saliva found its widest use at the race track where the saliva test for drugs became the determinant of a "fixed" horse race.[8] Saliva has proved to be of value in the confirmation or rejection of self-reports of cigarette usage among children and adolescents because there is present high concentration of thiocyanate in saliva of smokers as compared to non -smokers .[9]

Saliva & Caries

A number of antimicrobial agents have been identified in human saliva. These are usually divided into nonimmune and immune (immunoglobulin) factors. A vast number of reports document how these factors alone or in combination affect cariogenic microorganisms, in particular mutans Streptococci.[10]Different saliva based caries activity test are :**Lactobacillus Colony Count Test**- It estimates the number of acidogenic and aciduric bacteria in the patient's saliva.[10]**Snyder Test**- The Snyder test measures the rapidity of acid formation when a sample of stimulated saliva is inoculated into glucose agar adjusted to pH 4.7 to 5 and with bromocresol green as color indicator. Indirectly, the test is also a measure of acidogenic and aciduric

bacteria.[10]**Reductase Test**: The test measures the rate at which an indicator molecule, diazoresorcinol, changes from blue to red to colorless or leukoform on reduction by the mixed salivary flora.[10]Other test Buffer Capacity Test, Fosdick Calcium Dissolution Test, S. Mutans Adherence Method, S. Mutans Dip-Slide Methods.

Saliva As A Diagnostic Tools For Periodontal Disease

The use of saliva as a diagnostic and monitoring method for periodontal disease has been increasingly studied.[3]Given the specificity and sensitivity for bone resorption, pyridinoline crosslinks, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP), represent a potentially valuable diagnostic aid for periodontal disease.[11] When a combination of the biochemical markers such as osteocalcin, collagenase, PGE2, ?-2 macroglobulin, elastase, and alkaline phosphatase was evaluated, increased diagnostic sensitivity and specificity values of 80 and 91%, respectively, were reported.[11]

Systemic Diseases

Coeliac disease - Serum IgA antigliadin antibodies (AGA) are increased in patients with coeliac disease and dermatitis herpetiformis. Measurement of salivary IgA-AGA has been reported to be a sensitive and specific method for the screening of coeliac disease In a more recent study, salivary IgA-AGA produced sensitivity of 60% and specificity of 93.3% in the detection of coeliac disease. In comparison, serum IgG-AGA produced excellent sensitivity (100%) but lower specificity (63.3%).[1]**Diabetes Mellitus:** Glucose concentration in saliva of diabetic patients was mildly increased compare to healthy population, but this difference was not statistically significant ($t = 0.451$, $p = 0.05$).[12] Some authors suggested that mean salivary glucose levels were found to be significantly elevated in both uncontrolled and controlled diabetics, as compared to healthy non-diabetics.[13] The EGF concentration was significantly lower ($p < 0.05$) for the diabetic patients compared to control patients. Reduced levels of salivary EGF in diabetic patients may contribute to the development of oral and systemic complications of diabetes, which may have future clinical applications.[14]**Parkinson's disease**- Salivary production in patients of the Parkinson group was significantly lower than in controls (0.68±0.26 mg vs. 1.27±0.65 mg, respectively; p=0.009). Salivary concentrations of sodium, potassium, chloride were higher but amylase was lower than in controls (p=0.02, p<0.001, p=0.003, p=0.04, respectively).[15]

Autoimmune Diseases-sjögren's Syndrome

Sjogren's syndrome(SjS) is an autoimmune exocrinopathy which includes complaints of oral and ocular dryness, a result of salivary and lacrimal dysfunction. From Luciferase Immunoprecipitation Systems (LIPS) testing anti-Ro60 autoantibodies were detected in the saliva of 70% (19/27) of SjS patients with 96% specificity. Positive anti-Ro60 autoantibodies were also found in 70% of the matched serum samples (96% specificity). LIPS detected Ro52 autoantibodies in the saliva and serum of 67% of SjS patients with 100% specificity.[16]

Infectious Diseases

Helicobacter pylori infection: Saliva samples were tested for the presence of H. Pylori DNA by polymerase chain-reaction (PCR) assay, and sensitivity of 84% was reported .1Salivary antibodies are secreted during the immune response to infectious agents. Surprisingly, detection of specific IgA to H. pylori could not distinguish between infected or noninfected individuals but detection of IgG could. A method has been proposed using a special device (OraSure; Epitope, Beaverton, OR), a swab to be rubbed on the gums to obtain a gingivalmtransudate, enriched in IgG . Sensitivities and specificities obtained in these studies tend to be low, rarely reaching 90%.[17]**Entamoeba histolytica-** Detection of salivary lectin antigen of E. histolytica for the diagnosis of amoebic liver abscess (ALA) with a sensitivity and specificity of 22% and 97.4% respectively was reported.[18]**Mycobacterium tuberculosis:** 98% detection rate of Mycobacterium tuberculosis was obtained by polymerase chain reaction (PCR) using mixed saliva, in contrast to a 17.3% detection rate by cultivation.[7]**Pneumococcal pneumonia:** The detection of pneumococcal C polysaccharide in saliva by ELISA may offer a valuable complement to conventional diagnostic methods for pneumococcal pneumonia. Detection of this antigen in saliva demonstrated a sensitivity of 55% and specificity of 97%. The positive and negative predictive values were 0.94 and 0.73, respectively.[1]

Malignancy

Salivary analysis may aid in the early detection of certain malignant tumors. Tumor markers that can be identified in saliva may be potentially useful for screening for malignant diseases. **Squamous cell carcinoma:** p53 antibody can be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection of, and screening for, this tumor. Investigators have found that 13% (3/23) of patients with oral squamous cell carcinoma had antibodies against p53 in the saliva, by ELISA.7 Elevated levels of salivary defensin-1 were found to be indicative of the presence of oral SCC. A high-positive correlation was observed between salivary defensin-1 levels and serum levels of SCC-related antigen ($r = 0.879$). Potential salivary RNA biomarkers were IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT, and in combinations yielded sensitivity (91%) and specificity (91%) in distinguishing squamous cell carcinoma.[6]**Breast cancer:** Elevated levels of recognized tumor markers c-erbb-2 (erb) and cancer antigen 15-3 (CA15-3) were found in the saliva of women diagnosed with breast carcinoma, as compared with patients with benign lesions and healthy controls. Senstivity and specificity of salivary c-erb B-2 protein were 87% & 65% respectiviely.[19]Ovarian cancer: CA 125 is a tumor marker for epithelial ovarian cancer. Elevated salivary levels of CA 125 were detected in patients with epithelial ovarian cancer as compared with patients with benign pelvic masses and healthy controls. Saliva demonstrated a somewhat lower sensitivity than serum (81.3% vs. 93.8%, respectively); however, the specificity and positive predictive value were higher for saliva vs. serum (88.0% vs. 59.8% and 54.2% vs. 28.8%, respectively.6Leukemia:The oral manifestation of leukemias occur early in course of disease & these oral features can at times act as a diagnostic indicatior . A rise in salivary amylase levels in leukemic patient has been reported(p<.01).[20]**Gastric carcinoma:** It has been suggested that salivary nitrate, nitrite, and Nitrosamine may be related to the development of Oral and Gastric Cancer. Higher levels of salivary nitrate and nitrite, and increased activity of nitrate reductase, were found in oral cancer patients compared with healthy individuals, and were associated with an increased odds ratio for the risk of oral cancer.[1] High levels of nitrate in the saliva might therefore be associated with carcinoma of the digestive tract, and there is evidence from several localities of an association of increased incidence of gastric and hepatic carcinoma with high nitrate intakes and high salivary concentrations of

nitrate.[9]

Viral Diseases

Hepatitis: Saliva has also been utilized to detect very low levels of antibodies to HAV associated with vaccine-induced immunity. Comparison of serum and saliva levels of infection and vaccine-induced HAV-specific IgG have demonstrated excellent agreement (sensitivity = 98.7%, specificity = 99.6%). In comparing oral fluids to serum, sensitivity and specificity of 100% for the detection of HB surface antigen and antibodies to HCV, respectively, were reported.[21]Comparing the detection of HbsAg in saliva with that in serum by means of a commercially available serological kit yielded a sensitivity of 92% and specificity of 86.8%.[1]**Measles, mumps, rubella** :The detection of antibodies in oral fluid samples from subjects having received a measles/mumps/rubella vaccine produced sensitivity and specificity of 97% and 100% for measles, 94% and 94% for mumps, and 98% and 98% for rubella, respectively, in comparison with detection of serum antibodies for these viruses.[21]About the suitability & potential of oral fluid (OF) to substitute serum in estimating measles IgG antibodies , during community surveys by comparing optical densities (OD) of measles IgG antibodies in OF & serum in asymptomatic children was analyzed & found that Sensitivity : 89.5%, Specificity: 90.6%, Correlation coefficient : 0.97.[22]**Dengue:** Salivary levels of anti-dengue IgM demonstrates sensitivity of 90.3% and a specificity of 92.0% and demonstrating that salivary IgM is a useful diagnostic marker for DEN infection. Detection of IgA in serum may be another feasible alternative for the diagnosis of DEN infection, with serum IgA found in 68 (94.4%) of the IgM-positive cases.[23]**Human papillomavirus-** Only 57% of these patients had detectable HPV-16 DNA in salivary rinses, a sensitivity of 32.6%. Specificity of 98.7% can be achieved with this technique. Technologies such as competitive PCR coupled with mass spectrometry have promise to yield more specificity with positive results of one-copy number of DNA over real-time PCR, but the issue of false-positive nonpathologic detection of HPV remains.[6]**Human immunodeficiency virus (HIV)**-As compared with serum, the sensitivity and specificity of antibody to HIV in saliva for detection of infection are between 95% and 100%.[1]Salivary diagnostic Kits available are Aware BSP with a sensitivity of 99.4% and specificity of 99.9%, Aware OMT rapid test used for assessing the detection of antibodies to HIV-1 and HIV-2 in human oral fluid specimens. The sensitivity and specificity of the rapid HIV test kit are 99.5% and 99.98%.The OraQuick ADVANCE Rapid HIV 1/2 antibody test is intended for use as a point of care test to aid in the diagnosis of infection with HIV-1 and HIV-2 with a sensitivity of 100% and the specificity of 99.87%.

Drug Monitoring

Similar to other body fluids (i.e., serum, urine, and sweat), saliva has been proposed for the monitoring of systemic levels of drugs.**Therapeutic Drugs monitored** are Antipyrine, Caffeine, Carbamazepine, Lithium,Diazepam,Cyclosporine, Digoxin, Ethosuximide, Irinotecan , Methadone , Metoprolol, Oxprenolol , Paracetamol, Phenytoin,Quinine, Procainamide , Sulfanilamide, Theophylline, Tolbutamide, Cisplatin **Drug Abuse/Recreational Drug** monitored are Amphetamines, Ethanol, Marijuana, Nicotine, Opioids, Phencyclidine, Barbiturates, Cocaine Benzodiazepine. [1]

Monitring Of Hormone Levels

Hormones whose salivary levels reflect serum levels are Cortisol, Progesterone, Aldosterone, I7beta-Estradiol, Dehydroepiandrosterone, Testosterone,5alpha-Dihydrosterone, Estriol,Estrone,Insulin,Melatonin.[8]

Heavy Metals Found In Saliva

Heavy metals such as lead and cadmium are important occupational toxins and can also be found in saliva. A study by P an reported a strong correlation between saliva and blood lead (correlation coefficient, r=0.72) among adult males over a wide range of blood lead measurements. Blood contamination of saliva during sampling can spuriously raise salivary lead levels because the lead level in whole blood is 2-6 times higher than that in saliva.Normal range of lead in serum & saliva are 5-15microgram/dl and 0.7-7.5 microgram/dl respectively.24Cadmium is another heavy metal that has been detected in the saliva. The secretion of cadmium into saliva seems to occur by passive diffusion.[24]

Saliva In Forensic Genetics

Due to the presence of significant numbers of nucleated buccal epithelial cells in saliva, it is an excellent source of DNA from which a genetic profile of the donor is relatively easily obtained. Approximately 0.5-1.0 ng of DNA is required for such analysis, which represents approximately 80-160 cells. There is currently no definitive test for the positive identification of saliva, although there are a number of substances present in. These include the enzymes alkaline phosphatase and α-amylase and the inorganic anions thiocyanate and nitrite.[6]. The starch-digesting enzyme amylase is present in high concentrations in human saliva. In forensic investigations, amylase activity testing has been used to locate saliva stains on surfaces for over three decades . Presently, a screening method based on amylase testing could be useful to lower the number of negative DNA samples from crime scenes. A positive amylase result is indicative, not conclusive, for human saliva. The correlation between amylase activity and amount of cells/DNA in saliva therefore needs to be investigated.[25]

Future Diagnostic Tool

Tag-It device is a DNA-based test to detect cystic fibrosis from a patient's blood or saliva. .The **AmpliChip P450** Genotyping Test is a DNA test that measures how quickly particular drugs are cleared from the body so that medication levels can be customized for individual patient .[6]**TRUGENE HIV-1** genotyping test determines if a patient has a drug-resistant form of the virus.[6]

Conclusion

Saliva is a complex and dynamic biological fluid which contains a myriad of compounds. The biochemical and physical chemical properties of these salivary components and their interaction, accomplish the numerous functions that saliva performs in the oral cavity. Nowadays many assays are available to analyse various salivary parameters, however, standardization of collection and storage methods is essential to obtain meaningful results. The last few years saliva has gained increasing scientific interest not only for the excretion of various compounds, e.g., drugs, pollutants, hormones into saliva, but also the well documented relation of saliva with bacterial, viral and systemic diseases. The relatively easy non-invasive nature of collection and the relationship of saliva with plasma levels make saliva an attractive diagnostic tool. Nowadays, state-of the art proteomic methods are applied to search for the complete saliva protein composition. In addition, high throughput RNA and protein microarrays will be valuable tools for the identification of diagnostic biomarkers for a variety of diseases or description of whole body physiological changes.[4]Right now saliva diagnostics is not as mature as blood-

based tests. In order for this area to evolve there needs to be further development of the mini-sensors systems. The infrastructure has to be put into place and the dental community would need to change its practice at some level and be open to this option of serving as a health-screening center in a new type of role.

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Source of Support : Nill, **Conflict of Interest** : None declared