Journal of Oral Health & Community Dentistry

REVIEW ARTICLE

Saliva: A Miraculous Biofluid for Early Detection of Disease

Saxena V[1], Yadav NS[2], Juneja V[3], Singh A[4], Tiwari V[5], Santha B[6]

ABSTRACT

Saliva, a multi constituent oral fluid has high potential for the surveillance of general health and disease. To reach the above goal through saliva based diagnostics, two requisites must be fulfilled as discovering biomarker for different diseases among the complicated components of saliva and advancing sensitivity and specificity of biomarkers through persistent development of technologies with collective efforts over several years. Saliva has been demonstrated to be a promising bodily fluid for early diagnosis of disease and salivary diagnostics has exhibited tremendous potential in clinical applications. This review presents an impression of the value of saliva as a credible diagnostic tool, the innovation of salivary biomarkers and development of salivary diagnostics in the future.

Keywords: Saliva, biofluid, health and disease, biomarker.

[1]Professor
Department of Public Health Dentistry
Peoples College of Dental Sciences,
Bhopal, Madhya Pradesh (India)

[2] Professor
Department of Prosthodontics
Peoples Dental Academy,
Bhopal, Madhya Pradesh (India)

[3] Reader
Department of Dental Anatomy and Dental Histology
Sudha Rustagi College of Dental Sciences and
Research, Faridabad, Haryana (India)

[4] Senior Lecturer
Department of Public Health Dentistry
Peoples College of Dental Sciences,
Bhopal, Madhya Pradesh (India)

[5] Senior Lecturer
Department of Public Health Dentistry
RKDF, Bhopal, Madhya Pradesh (India)

[6] PG Student
Department of Public Health Dentistry
Peoples College of Dental Sciences,
Bhopal, Madhya Pradesh (India)

Contact Author

Dr. Vrinda Saxena
dr.vrinda@gmail.com

J Oral Health Comm Dent 2013;7(1)64-68

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 common easily accessible oral fluid, saliva plays a central role in several legends and anecdotes and contains information about the physiological states of the body. Saliva has hundreds of components which help detect systemic diseases and also provide biomarkers of health and disease status (1).

Saliva has three major functions: digestion, protection and lubrication. It also functions in maintenance of tooth integrity, is a good indicator of the plasma levels of various substances such as hormones and drugs and also the most available and non-invasive biofluid of the human body. It permanently "bathes" the oral cavity and copes with an ever-changing milieu. The oral cavity is a very complex and unique milieu due to its dual function. Gland-specific saliva can be used for diagnosis of pathology, specific to one of the major salivary glands. Whole saliva, however, is most frequently used for diagnosis of systemic diseases (2,3).

EARLY LANDMARKS 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. 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. The earliest sialochemical studies on oral fluids were conducted by Michaels and Kirk in the 1900's, each of whom examined saliva for specific 112 components that would be diagnostic for various systemic conditions. Standard collection techniques were established to minimize physiological variations.

NEED FOR SALIVARY DIAGNOSTICS

As a diagnostic fluid, saliva offers a distinctive advantage over serum. With new techniques for detecting small quantities of salivary components, including protein and messenger RNA, the field of salivary diagnostics has emerged as most promising area of research and need of the hour (3). 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.

Salivary diagnostic technology is showing promise in detection and monitoring malignancies at early stage and other various health concerns. The advantages of salivary diagnostics include ease of collection, elimination of fear of needles, inexpensive testing and reduced risk of disease transmission between healthcare workers and patients. In addition, salivary diagnostic is fast, highly sensitive and specific, portable, user-friendly and can screen for multiple agents simultaneously contributing in determining the scope of toxin exposure, thereby providing a quicker fight and diagnosis in the event of pandemic, epidemic and bioterrorism.

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 (3, 4).

SALIVA- MIRROR OF THE BODY

Oral fluid/Saliva, called the 'mirror of the body', is a perfect medium to be explored for health and disease. Saliva includes a large number of inorganic and organic compounds, which act as a "mirror of the body's health." In addition to its other functions, saliva could constitute the first line of defense against oxidative stress. Due to its composition and functions, saliva could have a significant role in controlling and/or modulating oxidative damages in the oral cavity. Saliva contains protein molecules which inhibit the growth of bacteria, prevent excessive swings in *p*H and begin the process of digestion. It's a mirror that reflects the levels of natural and artificial substances, indicates emotional and hormonal status, the health of the immune system, neurological conditions, nutritional deficits and metabolic states (2,4).

SALIVA A WINDOW TO THE BODY IN HEALTH AND DISEASE

The capability to assess physiological states, detect morbidity, initiation, progression and monitor post treatment therapeutic outcomes through a non invasive approach is one of the most desirable goals for health care research and delivery. Qualitative changes in salivary composition can also provide diagnostic information concerning oral as well as systemic predicament.

In the last few decades, there has been a focus on the utilization of saliva for bacteriological tests that give an indication of dental caries risk, salivary proteins interfere with bacterial colonization and also promote colonization. These proteins influence the enamel demineralization-remineralization process and dental caries formation (4). Furthermore, it has been demonstrated that functional formation of heterotypic complex between salivary molecules such as MG-1 (high-molecular-weight mucin glycoprotein-1), Amylase, PRPs (acidic prolin-rich protein-1), and Statherin are determination for plaque formation and dental caries. In addition, genetic factors should be included in associated with phenotypic expression of these proteins in mixed saliva, which may contribute to oral disease etiology (2-4).

SALIVA AND ABH BLOOD GROUP

The carbohydrates of salivary glycoprotein carry the ABH blood group antigens, expressing in the salivary glands and secreting in saliva. The expression of the blood group antigens in saliva may change the specific interaction between microorganisms and their salivary glycoprotein receptors, which might interfere in development and prevention of oral infectious diseases. This finding could be used for further researches on the relationship between the blood group antigens and oral diseases as well as on its forensic applications (5).

INFECTIOUS DISEASE

The use of saliva as a diagnostic and monitoring method for periodontal diseases has been increasing studied. Todorovic et al. analyzed the saliva of patients with periodontitis and demonstrated significant increases in enzyme activity in association with cell injury and tissue cell death, salivary enzyme activity, as biochemical markers, may be useful in diagnosis, prognosis, and monitoring of periodontal diseases (6,7). The levels of amylase and secretary IgA were significantly higher in whole saliva of subjects with periodontitis than in healthy controls. Uric acid levels are significantly lower in saliva from heavy smokers, oral cancer, periodontitis and diabetic patients with periodontitis, giving emphasis of oxidative stress in oral cavity. A lower level of saliva albumin was found in periodontal disease and diabetic patients, probably due to oxidative stress in the oral cavity (7).

Salivary peroxidase is lower in smokers, most probably due to the cyanide ions present in cigarette smoke, a very powerful inhibitor of hem peroxidase and in saliva from patients with oral cancer (8).

Mycobacterium tuberculosis is detected in oral fluid by PCR only when this infectious disease is in an acute phase and the level of bacteria is very high. Mycobacteria appear in the oral cavity in a great number. The diagnosis of streptococcal pneumonia and detection of pneumococcal C-polysaccharides are possible in saliva without detection of nucleic acids (9). The use of saliva as a diagnostic tool for Helicobacter pylori infection is an attractive option for epidemiologic studies when the non-invasive nature of the test is considered. DNA in the mouth using biological markers found in saliva.

Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva might, therefore, be a prognostic indicator for the progression of HIV infection. In conclusion, collection and analysis of saliva offer a simple, safe, well-tolerated, and accurate method for the diagnosis of HIV infection. The non-invasive nature in which a sample is collected eliminates the risk of infection inherent in collecting blood samples (9,10).

Regarding the certain viral diseases, detection of hepatitis A antigen and hepatitis B surface antigen in the saliva has been used

in epidemiological studies of to both types of hepatitis. Analysis of saliva provided a highly sensitive and specific method for the diagnosis of viral hepatitis B and hepatitis C.

Saliva has been used to detect antibodies against rubella, parotiditis and rubeola viruses. Saliva may also been used for determining immunization and detecting infection with measles, mumps, and rubella(9).

Another infectious disease of the oral cavity that can be diagnosed by saliva is candidiasis through the presence of Candida species in saliva (10). The detection of pneumococcal C polysaccharide in saliva by ELISA may offer a valuable complement to conventional diagnostic methods for pneumococcal pneumonia. Specific antibody to Taenia solium larvae has been demonstrated in saliva for detection of neurocysticercosis. It was suggested that saliva could be used in epidemiologic studies of this disease(11).

HORMONAL ANALYSIS

Saliva contains some free hormones that can be easily measured to show their availability in human tissue and to evaluate endocrine function. Hormones variation in saliva can be an indicative of cancer progress or the possibility of a disease like Cushing syndrome and Addison, Physical exercise and Stress (cortisol) (12). Primary aldestronism or Conn syndrome (aldosterone), Testicular function and behavioral studies of aggression, Depression, abuse, Violent and antisocial behavior (testosterone and dehydroepiandrosterone) fetoplacental function (estriol), Prediction of ovulation and ovarian function (progesterone).

Hormones commonly present in saliva are Androstenedione, Dihydrotestesterone, estradiol and insulin. Saliva is being used to detect a specific estrogenic hormone, estradiol, which has been found to predict preterm labour (13).

CARDIOVASCULAR DISEASES

Salivary endothelin concentrations are elevated in persons with chronic heart failure, and these levels can be used to assess disease severity. Salivary TC, TG, LDL-C and VLDL-C concentrations were significantly high in patients with ischemic stroke Cardiovascular diseases are a leading cause of death all over the world. Enzymes found in saliva, such as amylase, have been used for post-operative control of patients who had cardiovascular surgery. Several studies found the direct relationship between raised levels of alpha amylase and heart rate which increases under stress (14).

AUTOIMMUNE DISEASES

Sjogrens syndrome is a chronic disease affecting the lachrymal, salivary and other exocrine glands. Some procedures for the diagnosis of Sjogrens syndrome include sialography, salivary scintiography, biopsies, and serological tests. These tests are invasive, expensive, and sometimes conclusive. Levels of amylase, carbonic anhydrase and phosphate decrease in saliva but levels of calcium and potassium are usually normal. Parotid saliva from patients with rheumatoid arthritis and Sjogrens syndrome contains higher level of multiple forms of tissue kallikrein (15).

CYSTIC FIBROSIS (CF)

Besides the increased visco-elasticity of saliva in cystic fibrosis patients, there are several electrolyte and protein concentration differences compared with healthy individuals. The salivary concentrations of sodium, phosphate, chloride, lipid, epidermal growth factor and prostaglandin E2 also increase which are believed to play an important role in protection against dental decay (16).

DIABETES MELLITUS

Insulin is able to stimulate salivation so in diabetes mellitus patients the salivary flow rate decreases. In this situation salivation is easy to stimulate. It should be noted that medications used in these patients can also be responsible for the decreased salivary flow rate. Albumin and IgG concentrations of non stimulated saliva are lower than healthy individuals. Patients with diabetes mellitus express higher levels of amylase and secretory IgA in whole saliva constituents (17).

ALCOHOLIC LIVER CIRRHOSIS

Parotid enlarges in 50% of the patients with alcoholic liver cirrhosis, which results a 50% reduction of the salivary flow rate and a reduction of salivary sodium, bicarbonate and chlorine concentrations. The total salivary protein concentration decreases as well (18).

EPILEPSY

Gingival hypertrophy can be observed in patients with epilepsy who take phenytoin due to increasing of collagen synthesis and accumulation of proteoglycans. These patients should have a high quality oral hygiene. IgA deficiency is another side effect of phenytoin, resulting in a decreased immunological defense. Cyclosporine A and nifedipine are not associated with similar effects (19).

BURNING MOUTH SYNDROME

This syndrome is relatively most common in post-menopausal women. Patients complain of oral pain and dry mouth. Dry mouth can be developed after taking some medications like antidepressants. However, their salivation can be easily stimulated mechanically and chemically. The total salivary protein concentration of stimulated saliva is lower than in control subjects, but the total mucin concentration is higher. Salivary potassium, chloride and phosphate concentrations are also increased in the patients (3,9,10,20).

KIDNEY DYSFUNCTION

Half of all hemodialysis patients complain of hyposalivation, changes in taste, ammonium smelling breath and oral mucosal pain. The total salivary protein, sodium and potassium concentrations are similar to the plasma. Salivary pH of these patients is significantly higher than the healthy controls, due to the significantly increased salivary urea concentration. Gonzalez et al. found that saliva can be a good tool for early detection of exposure to lead and cadmium since salivary levels of these elements arise from the diffusible fraction of plasma (21).

SALIVA AS A DIAGNOSTIC TEST FOR DRUGS

Diagnostic testing of drugs using saliva/ oral fluid is now widespread and replacing

the previously used urine. Saliva is used to measure the level of Lithium, Carbamazepine, Barbiturates, Benzodiazepines, Phenytoin, Theophylline and Cyclosporine, Antipyrine, Caffeine, Cisplatin, Diazepam, Digoxin, Ethosuximide, Irinotacan, Methadone, Methoprolol, Oxpernolol, Parecetamol, Primidone, Procainamide, Quinine, Sulfanilamide, Tolbutamide and for drug abuse such as Amphetamine, Benzodiazepines, Cocaine, Ethanol, Marijuana, Nicotine, Opioids and Phencyclidine (22).

SIALOCHEMISTRY ANALYSIS

Researchers found that saliva can be a good tool for early monitoring of an exposure to lead and cadmium, because of higher salivary levels of these elements (9, 18) Sialochemistry of environmental heavy metals (cadmium, lead, mercury) may be useful in monitoring environmental, atmospheric and occupational pollutants.

SALIVA TELLS A TALE

Malignant tumors of the oral cavity

In case of primary oral tumors and recurrence measuring the level of selected biomarkers is adequately sensitive and specific monitor. Saliva much has also been considered in the detection of DNA biomarkers to diagnose spinocellular carcinoma of the oral cavity. Mutation of the tumor suppressor gene p53 is common in many malignancies. Elevated levels of salivary defencine-1 were found to be indicative of the presence of oral squamous cell carcinoma (19). Oral fluid is also used in diagnosing of other malignancies. Breast cancer is one of the first malignant tumors detected with the help of genetic protein biomarkers. Streckfus et al. drew attention to raised levels of CA15-3, epidermal growth factor (EGF) receptor and c-erb B2 in patients with breast cancer while Di-Xia, Schwartz, and Fan-Qin described elevated levels of CA125 and the glycoprotein complex in saliva of patients with ovarian cancer. Recent report suggests that head and neck carcinomas can be detected by utilizing DNA derived from the exfoliated oral mucosa cells collected in saliva. Franzmann et al. used CD44 protein in saliva as a potential molecular marker in diagnosing of head and neck cancer in all stages (20,23). In the presence of oral cavity malignancies the level of carcino embryonic antigen (CEA) in saliva increases, while the level of gastrointestinal cancer antigen decreases.IL-8 and thioredoxin can be used in the diagnosis of spinocellular carcinoma.

HEMATOLOGICAL ONCOLOGY

Wright et al. sought to establish high level of neutrophils in oral fluid that potentially indicated the success of bone marrow transplant. They detected neutrophils in saliva 2-3 days earlier than in the peripheral blood. In healthy people, they found diurnal fluctuating levels of neutrophils in saliva. Researchers also examined the importance of oral mucositis after myoablative chemotherapy and found that improvement was related to the appearance of neutrophils in the saliva. Lieschke et al. pointed to a steep rise in neutrophils after administering growth factors (G-CSF) in both blood and saliva which again proves their interdependence (20,23).

SALIVA AS A BIO MARKER

Salivary diagnostics could dramatically change the clinical practice by introducing point-of-care testing and real-time disease surveillance. Like blood, saliva contains many protein and RNA molecules, both of which are encoded by genes.

In the eyes of oral biologists, the functional value of saliva has long overshadowed the diagnostic possibilities. More recently scientists, a conservative lot, are slowly transitioning from viewing saliva as a diagnostic outcast in comparison with blood or urine, and are starting to view it as abundant, valuable scientific resource. However there may be cultural perceptions that block professional acceptance of salivary diagnostics and those can be overcome slowly. A drop of saliva harbours a world of diagnostic information, proteomically and genomically. A handful of these analytes mark human diseases with great sensitivity and specificity (20,23).

WRAPPING UP

A major draw back to use saliva as a diagnostic fluid has been the notion that informative analytes are generally present in lower amount in saliva than in serum.

With the new and very sensitive techniques, the lower level of analytes in saliva is no longer a limitation. Few road blocks hold back the realization of clinical diagnostic potential simple and inexpensive sampling methods that cause minimal subject discomfort. Definitive disease associated protein and genetic marker an accurate, portable and easy to use diagnostic platform.

NEXT STEP

As we enter the era of genomic medicine therefore salivary diagnostics will play an increasingly important role in the early detection of disease, the monitoring of disease progression, and the evaluation of patient behavior including treatment compliance and lifestyle choice. However, about the Sialochemistry in the twentieth century the best conclusion is perhaps, "Salivary Diagnosis: Promise for a new era in the field of diagnosis and to diagnose below the ice burg.

REFERENCES

1. Miller CS, Foley JD, Bailley Al, Campbll CL, Humphries RL, Christodoulides et al. Current developments in salivary diagnostics. *Biomark Med* 2010;**4**(1):171-189.
2. Tiwari M. Science behind human saliva. *J Nat Science Biol Med* 2011;**2**(1):53-58.
3. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: a review. *J Dent* 2005; **33**(3):223-33.
4. Carmen Llena Puy. The role of saliva in maintaining oral health and as aid to diagnosis. *Med Oral Poatol Oral Cir Bucal* 2006;**11**:449-55.
5. W Kim, YK Kim, SC Chung, SW Lee, HS Kho. Detection of ABH blood group antigens in the saliva of Koreans and their stability according to storage of saliva samples. *Forensic Science International* 2002;**129**:58-63.
6. Todorovic T, Dozic I, Vicente-Barrero M, Ljuskovic B, Pejovic J, Marjanovic M *et al.* Salivary enzymes and periodontal disease. *Med Oral Patol Oral Cir Bucal* 2005;**11**:115-19.
7. Totan A, Greabu M, Totan C, Spinu, T. Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase: possible markers in periodontal diseases. *Clin Chem Lab Med* 2006;**44**(5): 612-15.

8. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. *J Clin Periodontol* 2002;**29**(3):189-94.
9. Eliaz Kaufman. The diagnostic applications of saliva. A review. *Crit Rev Oral Biol Med* 2002;**13**(2):197-212.
10. Lima DP, Garcia DG, Saliba SA, Sumida DH, Okamoto AC. Saliva: reflection of the body. *Int J of Infect Dis* 2010;**14**(3):184-88.
11. Krook A, Fredlund H, Holmberg H. Diagnosis of pneumococcal pneumonia by detection of antigen in saliva. *Eur J Clin Microbiol* 1986;**5**:639-42.
12. Gafni RI, Papanicolaou DA, Nieman LK. Nighttime salivary cortisol measurement as simple, noninvasive,outpatient screening test for Cushing's syndrome in children and adolescents. *J Pediatrics* 2000;**137**:30-35.
13. HF Voss. Saliva as a fluid for measurement of estriol levels. *Am J Obstet Gynecol* 1999;**180**:S226-S31.
14. Denver RA, Tzanidis A, Martin P, Krum H. Salivary endothelin concentrations in the assessment of chronic heart failure. *Lancet* 2000;**355**:468-69.
15. Gotoh S, Watanabe Y, Fujibayashi T. Validity of stimulated whole saliva collection as a sialometric evaluation for diagnosing Sjogren's syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;**99**:299-302.
16. JKM Aps, J Delanghe, LC Martens. Salivary electrolyte concentrations are associated with cystic fibrosis transmembrane regulator genotypes. *Clin Chem Lab Med* 2002;**40**(4):345-50.
17. PA Moore, J Guggenheimer, KR Weynant, T Orchard. Type I diabetes mellitus, xerostomia and salivary flow rates. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontal* 2001;**92**:281-91.
18. Silvia Chiappin, Gioregia Antonelli, Rosalba Gatti, Elio F. De Palo Saliva specimen: A new laboratory tool for diagnostic and basic investigation. C*linical Chimia Acta* 2007;**383**:30-40.
19. M Hakeberg, LRM Hallberg, U Berggren. Burning mouth syndrome: experiences from the perspectives of female patients. *Eur J Oral Sci* 2003;**111**:305-11.
20. Herenia P Lawrence. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J Can Dent Assoc* 2002;**68**(3):170-74.
21. SM Shasha, H Ben Aryeh, A Angel, D Gutman. Salivary content in hemodialysed patients. *J Oral Med* 1983;**38**:67-70.
22. Drobitch RK, Svensson CK. Therapeutic drug monitoring in saliva. An update. *Clin Pharmacokinet* 1992;**23**:365–79.
23. Warnakulasuriya S, Soussi T, Maher R, Johnson N, Tavassoli M. Expression of p53 in oral squamous cell carcinoma is associated with the presence of IgG and IgA p53 autoantibodies in sera and saliva of the patients. *J Pathol* 2002;**192**:52-57.