(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

REVIEW ARTICLE

SALIVARY BIOMARKERS AS A DIAGNOSTIC INDICATOR - A BRIEF REVIEW

Jaskaranpreet Kaur Gill, Manjot Kaur, Kirtan Kaur, Sofia Goel

BDS, Sri Guru Ram Das Institute of Dental Sciences & Research, Amritsar, Punjab

ABSTRACT:
This review highlights the diagnostic application of saliva in health and disease. As a diagnostic fluid saliva offers distinctive advantages over serum as it can be collected non – invasively by individuals with modest training. Whole saliva is frequently used for diagnosis of systemic disease; as it is readily collected and contains serum constituent. Human saliva is not just the fluid in our mouth, but it mirrors our body's health and well being. Saliva has protective properties and contains a variety of antimicrobial constituents and growth factors. It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the old and infirm.
Key Words: Salivary diagnostic, biomarkers, systemic disease.

Corresponding Author: Jaskaranpreet Kaur Gill, Sri Guru Ram Das Institute of Dental Sciences & Research, Amritsar, Punjab, India, Email: gilljaskaran93@gmail.com

This article may be cited as: Gill JK, Kaur M, Kaur K, Goel S. Salivary biomarkers as a diagnostic indicator - A Brief Review. J Adv Med Dent Scie Res 2017;5(1):87-91.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2017.5.1.18

INTRODUCTION

Saliva, lacks the drama of blood, the sincerity of sweat and the emotion of the tears butyet is great importance to the body.[1]Saliva is a unique fluid, and interest in it as a diagnostic medium has advanced exponentially in the last decade. Saliva harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources. Although saliva reflects the body's health and well-being, its use as a diagnostic fluid has been hindered, mainly because of lack of understanding of the biomolecules present in it and their relevance to disease etiology, combined with the lack of high-sensitivity detection systems as a diagnostic medium.[2]

There are 4 main types of salivary glands in the mouth: submandibular, sublingual, parotid, and minor glands. The type of saliva that each gland produces reflects its rheological properties. For example, the parotid gland produce saliva that is identical to water (viscosity of parotid gland saliva approximately 1–3 mPa with low concentrations of secreted protein.[2,3] In addition to the molecules synthesized in the salivary glands [for example, mucins, cystatins and proline-rich peptides (PRPs)], saliva also contains molecules that are present in blood.[2]

Saliva can be considered as gland specific saliva and whole saliva. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland specific pathology, infection and obstruction.Whole saliva is most frequently studied when salivary analysis is used for the evaluation of systemic disorders. Saliva can be collected with or without stimulation.Stimulated saliva is collected by masticatory action or by gustatory stimulation. Unstimulated salivary flow rate is most affected by the degree of hydration.The best method to collect saliva is the draining method in which saliva is allowed to drip off.[4]

Salivary glands are composed of specialized epithelial cells and the basic secretory units of salivary glands are clusters of cells called acini. These cells can be classified as serous cells which secrete watery fluid that is essentially devoid of mucins and mucous cells which produce a very mucin rich secretion. The acinar cells secrete a fluid that contain water, electrolytes, mucus and enzymes all of which flow out of the acinus into collecting ducts. Acinar cells also produce and secrete alpha-amylase an enzyme that breaks down starch into glucose.[2,5] Through this review we would be highlighting the importance of saliva as a diagnostic marker in state of various pathologies.

BIOMARKERS IN SALIVA

Each salivary gland is highly permeable and enveloped by capillaries. This allows for the free exchange of blood-based molecules into the adjacent saliva producing acinus cells. It was postulated that blood-derived molecules entering salivary tissues via transcellular [passive and active transport] or paracellular [extracellular ultrafiltration] routes potentially influence the molecular constituency of oral fluids. The circulating biomarkers of disease potentially influence the biochemical composition of salivary secretions. Consequently, saliva may contain molecular information capable of communicating an individual's current state of health.[6]

SALIVARY BIOMARKERS

A biomarker is an objectively measured and evaluated indicator of normal biologic processes,pathogenic processes or pharmacological responses to therapeutic intervention.[7]Biomarkers are entities within the body capable of providing impartial information regarding the current physiologic state of a living organism.[8] Biomarkers exist in a variety of different forms,including antibodies, microbes, DNA, RNA, lipids, metabolites, and proteins. Alterations in their concentration, structure, function, or action can be associated with the onset, progression or even regression of a particular disorder resulting from how the body responds to it.[9]

In order for a biomarker to be used in a clinical assay, following milestones must be achieved:

- During preclinical testing, biomarkers must be developed using patient samples and confirmed at the in vitro and in vivo level.
- During the feasibility analysis, biomarkers must be tested using small patient subpopulations to demonstrate their ability to discriminate diseased from healthy subjects.
- During the validation process, biomarkers must be assayed accurately.
- Statistical analysis must be done to evaluate the discriminatory accuracy of the biomarkers in a large patient population.[6,10]

SALIVA VERSUS BLOOD

Saliva has many advantages over blood, which are:

- Collection is undemanding. While blood sampling requires highly trained personnel, saliva procurement can be done by anyone, including self-collection.
- The procedure is non-invasive.
- The samples are safer to handle.[11]
- Samples are easier to ship and store. Saliva does not clot and requires less manipulation than blood.
- The procedure is economical.

The correlation between salivary and blood-based constituents implies that although these two biofluids are separate and unique, they may be linked on a molecular level.[6]Inspite of these favorable attributes, use of saliva as a diagnostic fluid is not a mainstream idea yet. This is so because the levels of most analytes detected in blood serum and also found in saliva, are substantially diminished.[12]

Microbial biomarkers

As proposed in literature[6] there are three major points to be taken into consideration while determining the efficacy of microbial salivary diagnostics.

1. In order for microbes to be considered disease specific biomarkers, they must be associated directly with, but not necessarily the cause of, the condition in question.
2. If microbial biomarkers truly reflect health status, their regression or eradication should coincide with a positive therapeutic outcome.
3. Whether microbial markers can be used to assess the risk of disease? If so, could a saliva-based microbial profile serve as a predictive indicator of disease, and is there a healthy profile to strive for?

SALIVA AS A BIOMARKER IN VARIOUS SYSTEMIC DISEASES

Cardiovascular diseases

Atherosclerosis is triggered by the presence of inflammation which results in deposition of lipids in the arterial walls and progressive narrowing of the arterial lumen. This condition might then culminate in acute myocardial infarction (AMI).[13] It is possible to detect cardiac troponin,a biomarker for the detection of AMI in saliva that is released in response to cardiac cell necrosis.[14]A group of salivary biomarkers can complement findings of an ECG following an AMI. These markers include C- Reactive protein (CRP), myoglobin and myeloperoxidase, which in combination with an ECG show a highly significant correlation with myocardial infarct patients as compared to healthy controls.[15]

CRP is an inflammatory mediator that is produced in response to acute injury or infection and can mediate an inflammatory response by triggering the complement cascade. The salivary CRP levels were found to correlate with plasma CRP levels.[13,16]

Elevated salivary lysozyme levels has shown a significant association with hypertension which is an early stage of cardiovascular disease.[15]

Renal disease

Salivary markers are associated with end stage of renal disease. The list of markers includes cortisol, nitrite, uric acid, sodium, chloride, ph, amylase and lactoferrin. Salivary phosphate has been successfully used as a clinical biomarker for hyper-phosphatemia which is an important contributor to cardiovascular calcification in chronic renal

failure. Evaluation of phosphate levels in saliva correlated positively with serum creatinine and the glomerular filtration rate.[13]
Test strips were used to monitor salivary nitrate and uric acid before and after hemodialysis. So a salivary test could be used by patients to decide when dialysis is required, thereby eliminating unnecessary visits to a clinical dialysis.[17]

Diabetes
It is relatively easy to measure salivary glucose, due to the multiple sources of glucose in the oral cavity, but the salivary glucose levels do not correlate with the blood glucose levels. A unique proteomic signature in saliva from Type-2 diabetics as compared to control saliva, with proteins showed 2-fold change. Many of these proteins were associated with metabolic and immune regulatory pathways.[18]

Hereditary diseases
Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults, which is considered a generalized exocrinopathy. The organs affected in cystic fibrosisare; sweat glands,the lungs and the pancreas. Elevation in electrolytes, urea and uric acid, total proteins and lipids were observed in the submandibular saliva of CF patients.
21-hydroxylase deficiency is an inherited disorder of steriodogenesis which leads to congenital adrenal hyperplasia.Early morning salivary levels of 17- hydroxyl progesterone determined by ELISA is an excellent screening test for the diagnosis of non- classic 21-hydroxylase deficiency,since the salivary levels accurately reflected serum levels of 17-OHP.[17]

Autoimmune diseases
Sjogrens syndrome (SS), a chronic autoimmune disease characterized by dysfunction of salivary and lacrimal glands, keratoconjuctivitis sicca and xerostomia. Interleukin 2 and 6 are found in levels significantly high in individual that suffers from this disease.[17]
Sialochemistry may be used to assist in the diagnosis of SS.A consistent finding is increased concentrations of sodium and chloride. Elevated levels of IgA, IgM, lactoferrin, and albumin, and decreased concentration of phosphate were also seen in saliva of patient with SS.[19]
Sarcoidosis, is an inflammatory disease of the lymph nodes, lungs, liver, eyes, skin, or other tissues. A decrease in secretion volume of saliva in addition to a reduction in the enzyme activity of alpha – amylase and kallikrin is seen in most of the patients diagnosed of sarcoidosis.[17]

Malignancy
The search for biomarkers for a variety of malignancies is in progress since decades. Once the biomarkers are detected in serum, the same biomarkers can be looked for in saliva. For malignancies having fewer symptoms like ovarian and pancreatic cancer, the biomarker detection has a great impact on the survival rates.[15]CA 125 is a tumor marker for epithelial ovarian cancer. Salivary levels of CA 125 were elevated in patients with epithelial ovarian cancer. A positive correlation was found between salivary and serum levels of CA 125.
p53 is a tumor suppressor protein produced in cells exposed to various types of DNA-damaging stress. Inactivation of p53 is common occurrence in development of human cancer. As a result, accumulation of inactive p53 protein is observed, which in turn lead to production of antibodies directed against this protein. These antibodies can be detected in in sera of patients with different types of malignancies.[20]
p53 antibody can be detected in the saliva of patient diagnosed with oral squamous cell carcinoma (OSCC) thus assisting in the early detection and screening. A high positive correlation was observed between salivary defensin -1 levels and serum of OSCC –relatedantigen.[21]

Oral diseases
The physicochemical and biochemical properties of saliva along with its complex composition gives saliva its anti-bacterial, anti-viral and anti-fungal properties; buffering capacity for plaque acids; digestive activity; mineralizing agents for protection and repair of hard tissues; lubricating property; and repairing fluid for mucosal surfaces.[15]

Dental caries
Caries is a result of demineralization of the tooth surface initiated by acid production of cariogenic bacteria. This process can ultimately lead to tooth loss. It is demonstrated in various studies that S. mutans initiates dental caries; while Lactobacilli have a role in progression of carious lesions. High salivary levels of both pathogens have shown a positive correlation with the presence of caries in both children and adults.[22]Saliva secretion rate and buffering capacity has proven to be sensitive parameters in caries production. High number of S. mutans, and Lactobacillus indicate a shift in oral microflora from healthy to cariogenic.[23]

Periodontal disease
Periodontitis is characterized by destruction of the periodontal tissues such as gingiva and bone that support the tooth. The activation of inflammatory mediators of host cells upon exposure to periodontal pathogens and their products primarily cause periodontitis. Various salivary biomarkers for periodontal diseases include inflammatory mediators, enzymes, epithelial keratins, immunoglobulins, salivary ions and hormones.[24]
As periodontitis is a multi-factorial disease, not only host-derived factors should be analyzed in saliva but also the oral pathogens. Higher salivary levels of *Porphyromonas gingivalis*, *Tannerella forsythis* and *Prevotella intermedia* are found in individuals with progressive periodontitis.[25]

Mutations in cathepsin –C gene has been identified as causal for Papillon –Lefevre syndrome. The loss of attachment and deepening of periodontal pocket leads to increased leakage of a serum like fluid designated gingival crevicular fluid into the oral cavity.[23]

Viral diseases

Saliva is found to be a useful alternative to serum for the diagnosis of viral hepatitis. Acute Hepatitis A (HAV) and Hepatitis B were diagnosed based on the presence of IgM antibodies in saliva.[26]Quantitative detection of DNA is used to evaluate the levels of virus in the body and also been used for screening hepatitis B surface antigen (Hbs Ag) in epidemiological studies.[27]

Patients with suspected HIV infections can now be screened for HIV-1 and HIV-2 via a enzyme linked immunosorbent assay [ELISA]. Although positive results may be confirmed with a follow-up Western Blot, this ELISA commonly generates accurate results rapidly and eliminates the necessity for invasive blood draws.[28]A swab is left in place for 2-5 minutes between the lower gingival and buccal mucosa to collect saliva. Then the swab is sent to lab for Western Blot analysis to confirm the diagnosis.[29]Diagnosis of infection with HIV based on specific antibody in saliva is equivalent to serum in accuracy, and therefore applicable for both clinical and epidemiological surveillance. Salivary IgA levels to HIV decline as infected patients become symptomatic. Therefore, detection of IgA antibody to HIV may be prognostic indicator for progression of HIV infection.[30]

Saliva may be used for determining,immunization and detecting infection with measles, mumps and rubella.Dengue is a mosquito transmitted viral disease. Salivary levels of anti-dengue IgM andIgG were demonstrated in diagnosis of infection.[4]The salivary IgA response was found to be a better marker of rotavirus infection than the serum antibody response.[31]

Bacterial Infections

Helicobacter pylori infection is associated with peptic ulcer disease and chronic gastritis.Infection with this bacterium stimulates the production of specific IgG antibody. H. pylori exists in higher prevalence in saliva than infaeces.[32]There is considerable variation in the detection rate of H. pylori DNA in salivary samples.[17]

Children infected with **Shigella** reveal higher titers of anti-lipopolysaccharide and anti-Shiga toxin antibody in comparison with healthy controls.[33]The detection of pneumococcal C polysaccharide in saliva by ELISA offers a valuable complement to conventional diagnostic methods for Pneumococcal pneumonia.[34]

Lyme disease is caused by the spirochete Borrelia burgdorferi and is transmitted to humans by blood-feeding ticks.The detection of anti-tick antibody in saliva has potential as a biomarker of exposure to ticks bites, which in turn may serve as a screening mechanism for individuals at risk for Lyme disease.[35]

Forensics

Salivary tests have been used for a wide variety of forensic studies. Samples can be obtained from drinking glasses, cigarette butts, envelops, and other sources and then used to detect blood-group substances or salivary genetic proteins [primarily proline-rich protein polymorphisms]. Saliva contains blood-group antigens A, B, H and Lewis antigens that are used for identification of individuals.

Deoxyribonucleic acid [DNA] and messenger ribonucleic acid [mRNA] are detected in saliva. PCR allows replication of thousands of copies of a specific DNA sequence in vitro, enabling the study of small amounts of DNA.[36]

CONCLUSION

Each of us has inside our mouth a key to the pathological and disease diagnostic biomarker library hidden inside our bodies. Saliva is the source to all this information. Salivary diagnostics is a dynamic field that is being incorporated as part of disease diagnosis, clinical monitoring and for making important clinical decisions for patient care.[37]

The ability to monitor health status, disease onset, progression, and treatment outcome through noninvasive means is a highly desirable goal in health care promotion and delivery. Saliva is a perfect medium to be explored for health and disease surveillance.Discovering, validating and understanding saliva based biomarkers could have a considerable role in establishing oral fluids as a credible diagnostic biofluid.

REFERENCES

1. ID Mandel. The diagnostic uses of saliva. JOral Pathol Med 1990;19:119-125.
2. Pfaffe T, White JC, Beyerlein P, Kostner K, Punyadeera C. Diagnostic Potential of Saliva: Current State and Future Appl. ClinChem 2011; 57(5):675-687.
3. Veerman EC, Valentin-Benz M, NieuwAmerongen AV. Viscosity of human salivary mucins: effect of pH and ionic strength and role of sialic acid. J BiolBuccale 1989;17:29**7-**306.
4. Kaufman E, Lamster IB. The diagnostic applications of saliva- A review. IntAmerAssoc Dent Res 2002; 13(2):197-212.
5. Kalk WW, Vissink A, Stegenga B, Bootsmaa H, NieuwAmerongen AV, Kallenberg CG. Sialometry and sialochemistry: a non-invasive approach for diagnosing Sjogren's syndrome. Ann Rheum Dis 2002;61:137-144.
6. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DTW. Salivary biomarkers: towards future clinical and diagnostic utilities. ClinMicrobiol Reviews 2013;26(4):781-791.
7. Silberring J, Ciborowski P. Biomarker discovery and clinical protomics. Trends Anal Chem. 2010;29:128-140.
8. Ilyin SE, Belkowski SM, Plata-Salaman CR. Biomarker discovery and validation: technologies and integrative approaches. Trends Biotechnol. 2004;22:411-416.

9. Wagner PD, Verma M, SrivastavaS.Challenges for biomarkers in cancer detection. Ann N Y Acad Sci. 2004;1022:9-16.
10. Bonassi S, Neri M, Puntoni R. Validation of biomarkers as early predictators of disease. Mutat Res 2001;480-481:349-358.
11. Campo G, Perea MA, Del Romero G, Cano J, Hernando V, Bascones A. Oral transmission of HIV, Reality or Fiction? An update. Oral Dis. 2006;12:219-228.
12. Miller S. saliva testing-a non-traditional diagnostic tool. Clin Lab Sci. 1994;7:3-44.
13. Khot UN, KhotMB ,Bajzer CT. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA. 2003;290(7):898-904.
14. Saunders JT, Nambi V, de Lamos JA. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure and mortality in the atherosclerosis risk in communities study. Circulation. 2011;123:1367-1376.
15. Malamud D, Rodriguez-Chavez IR. Saliva as a diagnostic fluid. Dent Clin North Am. 2011;55:159-178.
16. Out D, Hall RJ, Granger DA, Page GG, Woods SJ. Assessing salivary C-reactive protein: longitudinal association with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. Brain Behav Immun. 2012; 26:543-551.
17. Arunkumar S, Arunkumar JS, Burde KN, Shakunthala GK. Developments in diagnostic applications of saliva in oral and systemic diseases- A comprehensive review. J Scient Inn Res 2014;3:372-387.
18. Rao PV, Reddy AP, Lu X, et al. Proteomic identification of salivary biomarkers of type-2 diabetes. J proteome Res. 2009;8:239-245.
19. Ben-Aryeh H, Spielman A, Szargel R, Gutman D, Scharf J, Nahir M, et al. sialochemistry for diagnosis of Sjogren's Syndrome in Xerostomic patients. Oral Surg Oral Med Oral Pathol. 1981;52:487-490.
20. Hainaut P, Vahakangas K. p53 as a sensor of carcinogenic exposures: mechanisms of p53 protein induvtion and lessons from p53 gene mutations. Pathol Biol. 1997;45:833-844.
21. Tavassoli M, Brunel N, Maher R, Johnson NW, Soussi T. p53 antibodies in the saliva of patients with squamous cell carcinoma of oral cavity. Int J Cancer. 1998;78:390-391.
22. Nishikawara F, Katsumura S, Ando A. Correlation of cariogenic bacteria and dental caries in adults. J Oral Sci. 2006; 48(4):245-251.
23. Morrison HI, Ellison LF, Taylor GW. Periodontal disease and risk of fatal coronary heart and cerebrovascular disease. J Cardiovasc Risk.1999;6:7-11.
24. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis- a review. J Clin Periodontal. 2000; 27(7):453-465.
25. Saygun I, Nizm N, Keskiner I. Salivary infectious agents and periodontal disease status. J Periodontal Res 2011; 46(2):235-239.
26. Parry JV, Perry KR, Pandey S, Mortimer PP. Diagnosis of hepatitis A and B by testing saliva. J Med Virol.1989;28:255-260.
27. Zhang YL , Pan HY , Chen CR , Lou GQ , Ye RX , Lu DR. The role of saliva testing for preventing hepatitis B virus spreading. Zhonghua Yu Fang Yi XueZaZhi. 2008;42;596-598.
28. Delaney KP , Branson BM , Uniyal A , Kerndt PR , Keenan PA , Jafa K , Gardner AD , Jamieson DJ , Bulterys M. performance of an oral fuild rapid HIV- 1/2 test: experience from four CDC studies. AIDS.2006;20:1655-1660.
29. Krishnamurthy S, Vasudeva SB, Vijayasarathy S. salivary gland disorders: A comprehensive review. World J Stomatol2015; 4 (2):56-71.
30. Friedman MG. Radioimmunoassay for the detection of virus-specific IgA antibodies in saliva. J Immunol Meth. 1982;54:203-211.
31. Aiyar J, Bhan MK, Bhandari N, Kumar R, Raj P, Sazawal S. Rota virus specific antibody response in saliva of infants with rotavirus diarrhea. J Infect Dis. 1990;162:1383-1384.
32. Li C, Ha T, Fergusan DA, Chi DS, Zhao R, Patel NR, et al. A newly developed PCR assay of H. pylori in gastric biopsy, saliva and feces. Evidence of high prevalence of H. pylori in saliva supports oral transmission. Dig Dis Sci. 1996;41:2142-2149.
33. Schultsz C, Qadri F, Hossain SA, Ahmed F, Ciznar I. Shigella specific IgA in saliva of children with Bacillary Dysentery. FEMS microbial immunol.1992;4:65-72.
34. Krook A, Fredlund H, Holmberg H. Diagnosis of pnumococcal pneumonia by detection of antigen in saliva. Eur J ClinMicrobiol 1986;5:639-642.
35. Schwartz BS, Ford DP, Childs JE, Rothman N, Thomas RJ . Anti-tick saliva antibody: A biologic marker of tick exposure that is a risk factor for Lyme disease seropositivity. Am J Epidemiol. 1991;134:86-95.
36. Sweet D, Hildebrand. Saliva from cheese bite yields DNA profile of Burglar: A case report. Int J Legal med.1999;112:201-203.

Corstjens PLAMMD. Point-of-care diagnostics for infectious diseases. Saliva diagnostics Ames:Wiley-blackwell;2008:243-254.

Source of support: Nil **Conflict of interest:** None declared