DOI:10.24041/ejmr2017.10

DIAGNOSIS IN A SPIT : A SHORT REVIEW

Sonia Jaiswal, Shirin Jahan *, P.K Sharma, R.K Srivastava*
Department of Anatomy
Era's Lucknow Medical College And Hospital, Sarfaraz Ganj, Lucknow, U.P., India.-226003
*Rama Medical College and Hospital, Rama University. Kanpur, U.P., India.-209217

ABSTRACT

Salivary diagnostics is a cost effective, minimally invasive procedure. This bio-fluid can be easily collected, transported and stored. There are a variety of conditions where saliva can be used as a diagnostic tool such as measurement of stress, enzyme levels, developmental disease biomarkers and cancer mutations. A number of studies have focused their attention on the assessment and monitoring of oncologic biomarkers in saliva. Modern techniques, chemicals and various equipment have made laboratory investigations of saliva easy and precise. Its clinical applications and analysis has made salivary diagnostics a useful tool in the field of Medicine and Dentistry. The methodology for diagnosis of oral and systemic diseases has been a subject of study by several researchers with the aim of increasing its use as a diagnostic tool.

Received on : 30-05-2017
Accepted on : 11-06-2017

Address for correspondence
Dr Sonia Jaiswal
Department of Anatomy
Era's Lucknow Medical College & Hospital, Lucknow-226003
Email:jaiswalsonia2008@gmail.com
Contact no. :+91-9839020777

KEYWORD: Salivary Diagnostics, Biofluid, Disease Biomarker.

INTRODUCTION

Saliva is a unique bio-fluid that has varied amount of proteins, polypeptides, nucleic acids, electrolytes, and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 7.2–7.4 (1). Saliva as a bio-fluid has been a subject of research for many years but interest in its biochemical and physicochemical properties gained impetus when researchers found that it 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 (2,3). The collection and analysis of saliva is non-invasive and cost effective. An understanding of the salivation mechanism coupled with advanced techniques and equipment can help in the confirmation of the molecular basis of many disorders. This in turn will open up the possibility of "point of care" diagnostics using the oral bio-fluid (4).

SALIVA V/S SERUM

Saliva has been identified to be functionally equivalent to serum. It reflects the physiological state of the body, including hormonal, emotional, nutritional, and metabolic variations (5). It is one of the easiest body fluids to collect as it is a simple, non-invasive chair side procedure and does not require any special equipment. It is cost effective and can be used as a diagnostic tool for mass screening purposes. There is a facility for repeated sampling in a short interval of time. The sampling procedure is safe for the operator and the patient. Saliva has thus been found to be effective as a diagnostic tool (6, 7).The linkage of saliva with traditional biochemical parameters which appear in the serum makes it an interesting diagnostic tool (8).

METHOD OF COLLECTION

Saliva can be collected either in stimulated or un-stimulated conditions. Un-stimulated saliva is collected by allowing saliva to collect in the mouth and then it is either drained in a wide bore sterile vessel or collected by using a cotton swab in the mouth or by using suction methods. Stimulated whole saliva is collected by asking the patient to chew paraffin wax. The same can also be collected by applying acetic acid in the mouth (9). The disadvantage of using stimulated saliva is that the bio fluid collected may not only be watery in secretion but will also have altered Ph. However, it is the method of choice when there is a decrease in salivary secretion. Recently new methods of collection of saliva have been introduced (10). Oragene is a commonly used sophisticated technique where preservation buffers are used to protect the sample until processing and extraction take place. Other methods of saliva expectoration include Saligene, Oracol, and Verofy.

SALIVAAS A DIAGNOSTIC TOOL

Role of saliva in oral diseases such as dental caries and periodontitis

There are a number of antimicrobial agents present in saliva which have been further divided into immune and non-immune factors. These factors affect the micro-organisms alone or in combination (11). There are different saliva based tests such as lactobacillus colony count test, which estimates the number of acidogenic and aciduric bacteria in the patient's saliva. Synder test and Reductase test are other tests which measure the rapidity of acid formation and measures the the colour change of diazoresorcinol on reduction by the salivary flora respectively.

Saliva based tests for periodontal diseases

Pyridinoline crosslinks, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP),represent a potentially valuable diagnostic aid for periodontal disease (12)

Diagnosis of infections

Oral fluids contain various biomolecules of which IgG is derived from the gingival crevicular fluid and the mucosal transudate. IgG levels in saliva are the antibodies used in the screening of viral infections and immunization. Viral lesions are associated with the shedding of the virus into the saliva (13). The salivary antibodies of human immunodeficiency virus (HIV),hepatitis C virus (HCV), hepatitis A virus (HAV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and rubella virus have a considerable correlation with those of the serum values. Salivary antibodies have also been found positive following immunization against poliovirus, rotavirus, and HIV (14).

Diagnosis of tumor

Saliva is an emerging diagnostic method in the diagnosis and prognosis of malignancies. Tumour markers like c-erb B2, p53, and CA125 which are used in screening and diagnosis of several malignancies. The genetic analysis and their expression levels can be determined by salivary RNA transcript with microarray analysis (15). Application of salivary diagnostics also includes the detection of ovarian cancers, breast cancers using tumour markers (16). 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. It has been suggested that salivary nitrate, nitrite, and Nitrosamine may be related to the development of Oral and Gastric Cancer (17).

Monitoring of drug levels

Saliva serves as a diagnostic tool in determination of various pharmaceutical drugs like lithium and digoxine phenol barbital which have a narrow therapeutic index. Further, alcohol, amphetamines, barbiturates, and opioids, which serve as commonly used abused drugs, can also be monitored using saliva (18,19).

An assessment of hormone levels

Saliva serves as a tool in determination of protein polypeptide hormones and non-peptide hormones (20). Hormones whose salivary levels reflect serum levels are Cortisol, Progesterone, Aldosterone, I7 beta-Estradiol, Dehydroepiandrosterone, Testosterone,5alpha-Dihydrosterone, Estriol, Estrone, Insulin, Melatonin (21).

Sialochemistry

Heavy metals such as lead and cadmium are important occupational toxins and can also be found in saliva**.** The presence of these heavy metals in saliva occurs by diffusion (18)

Diagnosis of autoimmune disorders

Sjogren's syndrome is an autoimmune endocrinopathy characterized by xerophthalmia, xerostomia, and keratoconjunctivitis. IgA secreted by the salivary gland serves as a marker in Sjogren's syndrome patients (22)

Saliva and wound healing

Saliva helps in wound healing apart from its role in preventing wound infections. The EGF present in saliva has angiogenic and proliferative effects which enhances the wound healing (23). Saliva also contains clotting factors like IXa, VIII, and XI. Increased level of salivary kallikrein has a major role in vasodilatation around mucosal injuries to facilitate defence and healing of injured areas (24).

Role of saliva in other systemic disorders

Acute stress conditions also lead to significant salivary changes with a prominent decrease in secretary IgA and increase in salivary amylase and molecular chaperoneHsp70. There is also a prompt change in the bacterial adherence to the mucins. Saliva has been used to assess the salivary creatinine levels in diagnosis and monitoring of the kidney failure (25). The presence of cardiac enzymes in saliva helps in obtaining promising results in the identification of acute myocardial infarction (26). Cystic fibrosis can be detected by measuring the lipid levels of submandibular saliva which is considerably raised leading to increased calculus formation. Abnormally elevated prostaglandins E2 and poor biologic activities of EGF are also key features of cystic fibrosis (25).

LIQUID BIOPSY AND CIRCULATING CELLS AND DNA

Saliva has been useful as a liquid biopsy tool in point of care diagnostics as it is a convenient method of diagnosing and identifying cancer types from biofluids, instead of using conventional biopsy of tissue sampleswhich is an invasive procedure. Circulating tumor DNA (ctDNA) is a constituent in saliva, which can be extracted or detected using specialized techniques. As opposed to the various purification techniques that require high sample volumes and specialized equipment, ctDNA targets can simply be isolated through traditional DNA extraction methods (27). On a clinical level, it should also be noted that ctDNA may also aid in other molecular diagnostics besides cancer. Circulating cell-free DNA can furthermore indicate several conditions such as autoimmune diseases, stroke, sepsis, trauma, and myocardial infarction (28, 29). Both circulating tumor cells and ctDNA targets for cancer profiling offer various advantages and disadvantages, and while there is no definitive answer regarding the future of liquid biopsy diagnostics, in general, it can be stated that ctDNA has occupied a dominant position in recent years since it is simpler to work with and does not require the usage of specific extraction methods such as beads or microfluidics to achieve separation from a biofluid sample (30).

Appearance of tumor markers in saliva

Although it is inevitable that the markers of oral cancer will be reflected in the saliva, the relationship between systemic disease/ cancer and appearance of biomarkers in saliva is yet unclear. Several intracellular and extracellular pathways enable the biomolecules to move from blood capillaries to the saliva (31) Many of the biomolecules enter into saliva from the blood by passing through the spaces between cells by transcellular (passive intracellular diffusion and active transport) or paracellular routes (extracellular ultrafiltration). These markers may be carried by the local capillaries of the

salivary glands to the oral cavity through the flow of gingival crevicular fluid (GCF) (17). In an experiment conducted on tumor bearing mice an altered salivary protein pattern was noted which confirmed the speculation of the researchers that the tumors secrete mediators which may affect the activity of transcription factor in salivary glands, thereby inducing either an up or down-regulation of protein transcript levels in saliva (32).

NEWER DIAGNOSTIC TECHNIQUES

MyPerioID and MyPerioPath are DNA based saliva tests to determine the type and concentration of bacteria that cause periodontal disease. My PerioID test also determines the genetic susceptibility to periodontal disease and identifies patients of risk (33). Oral fluid nano-sensor test is a micro electromechanical system that is capable of real time, ultrasensitive, ultra specific detection of salivary protein and RNA biomarkers (34). This envisioned product has a great value in multiplex detection of salivary biomarkers for oral cancer patients. It also analyses saliva for the presence of salivary mRNA biomarkers (SAT,ODZ, IL-8, and IL-1b) and two salivary proteomic biomarkers(thioredoxin and IL-8). Ora Quick is an antibody test which detects the HIV1 and HIV2 in the saliva, serum, and plasma and is a quick chair side test which provides results in 20 minutes. Integrated microfluidic platform for oral diagnostics (IMPOD) is a point of care diagnostic test which enables rapid quantification of a biomarker associated with the oral disease from the saliva. It is one of the best methods for detection of MMP8 and other biomarkers in small concentrations of saliva (35). Integrating the new salivary diagnostic methods into clinical practice will aid the clinicians in making health related decisions for the patients.

CONCLUSION

Saliva is a complex and dynamic biological fluid which contains a variety of compounds. Salivary diagnostics is an emerging field of research. Biomarkers found in saliva comes from the the gingival crevicular fluid and mucosal transudate which infiltrate the saliva due to oral and systemic diseases. Nowadays many assays are available to analyse various salivary parameters, however, standardization of collection and storage methods is essential to obtain meaningful results. The relatively easy non-invasive nature of collection and the relationship of saliva with plasma levels make saliva an attractive diagnostic tool. Type of saliva; stimulated or unstimulated will have a significant effect on salivary biomarker composition and analysis and this needs to be taken in to consideration when using saliva for diagnostic/ screening purposes. Future developments in salivary diagnostics for screening, risk assessment and therapeutic management for a range of conditions are promising and this approach will hopefully allow individualized treatments before arise of major clinical symptoms

REFERENCES

1. J. L. Chicharro, A. Luc´ıa,M. P´erez, A. F. Vaquero, andR.Ure˜na,"Saliva composition and exercise," Sports Medicine, vol. 26, no.1, pp. 17–27,1998.
2. Lima DP et al. Saliva: reflection of the body. International Journal of Infectious Diseases 2010;14: e184-e188.
3. Schipper RG, Silletti E, Vingerhoeds MH. Saliva as Research Material: Biochemical, Physicochemical and Practical Aspects. Archives of oral biology 2007; 52:1114 - 1135.
4. Wong DT. Salivary Diagnostics, ed-1st, Wiley-Blackwell 2008.
5. Y. H. Lee andD. T.Wong, "Saliva: anemerging biofluid for early detection of diseases," American Journal ofDentistry, vol. 22,no.4, pp. 241–248, 2009.
6. D. Malamud, "Saliva as a diagnostic fluid," Dental Clinics of North America, vol. 55, no. 1, pp. 159–178, 2011.
7. C. F. Streckfus and L. R. Bigler, "Saliva as a diagnostic fluid," Oral Diseases, vol. 8, no.2, pp. 69–76, 2002.
8. C. Silvia, A. Giorgia, G. Rosalba, F. Elio, and P. De, "Saliva specimen: a new laboratory tool for diagnostic and basic investigation,"Clinica Chimica Acta, vol. 383, no. 1-2, pp. 30–40, 2007.
9. M. Navazesh, "Methods for collecting saliva," Annals of the New York Academy of Sciences, vol. 694, pp. 72–77, 1993.
10. S. G. Oberg, K. Izutsu, and E. L. Truelove, "Human parotid saliva protein composition: dependence on physiological factors," The American Journal of Physiology, vol. 242, no. 3, pp. 231–236, 1982.
11. Prabhakar AR et al. Diagnostic Applications of Saliva in Dentistry. International Journal of Clinical Pediatric Dentistry 2009; 2(3):7-13.
12. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontology2000 2009; 50:52-64.
13. L. Samaranayake, "Saliva as a diagnostic fluid," International Dental Journal, vol. 57, no. 5, pp. 295–299, 2007.
14. S. Chiappin, G. Antonelli, R. Gatti, and F. D. de Palo, "Saliva specimen: a new laboratory tool for diagnostic and basic investigations," Clinica Chimica Acta, vol. 383, no. 1-2, pp. 30–40, 2007.
15. B. P. Priti and R. P. Basgauda, "Saliva: a diagnostic biomarker of periodontal diseases," Journal of Indian Society of Periodontology, vol. 15, no. 4, pp. 310–317, 2011.
16. M.-S. Kang, J.-S. Oh, H.-J. Kim et al., "Prevalence of oral microbes in the saliva of oncological

patients," Journal of Bacteriology and Virology, vol. 39, no. 4, pp. 277–285, 2009.
17. Kaufman E, Lamster IB. The Diagnostic Applications of Saliva: a Review. Crit Rev Oral Biol Med 2002; 13(2):197-212.
18. S. Chiappin, G. Antonelli, R. Gatti, and F. D. de Palo, "Saliva specimen: a new laboratory tool for diagnostic and basic investigations," Clinica Chimica Acta, vol. 383, no. 1-2, pp. 30–40,2007.
19. A. H. Garde and A. M. Hansen, "Long-term stability of salivary cortisol," Scandinavian Journal of Clinical and Laboratory Investigation,vol. 65, no. 5, pp. 433–436, 2005.
20. D. L. Mager, A.D. Haffajee, P. M. Delvin, C. M. Norris, M. R. Posner, and J. M. Goodson, "The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects," Journal of Translational Medicine, vol. 3, article 27, 2005.
21. Mandel ID: The diagnostic Uses of Saliva. J Oral Pathol Med 1990; 19: 119-25.
22. E. A. Shirtcliff and R. T. Marrocco, "Salivary cotinine levels in human tobacco smokers predict the attentional validity effect size during smoking abstinence," Psychopharmacology, vol. 166, no. 1, pp. 11–18, 2003.
23. T. Zelles, K. R. Purushotham, S. P. Macauley, G. E. Oxford, and M. G. Humphreys-Beher, "Saliva and growth factors: the fountain of youth resides in us all," Journal of Dental Research, vol. 74, no. 12, pp. 1826–1832, 1995.
24. A. S. V. Burgen and N. G. Emmelin, Physiology of the Salivary Glands, Edward Arnold, London, UK, 1961.
25. L. Heflin, S. Walsh, and M. J. Bagajewicz, "Design of medical diagnostics products: a case-study of a saliva diagnostics kit," Computers and Chemical Engineering, vol. 33, no. 5, pp. 1067–1076, 2009.
26. P. N. Floriano, N. Christodoulides, C. S. Miller et al., "Use of saliva-based nano-biochip tests for acute myocardial infarction at the point of care: a feasibility study," Clinical Chemistry, vol. 55, no. 8, pp. 1530–1538, 2009.
27. Jen J, Wu L, Sidransky D. An overview on the isolation and analysis of circulating tumor DNA in plasma and serum. Ann N Y Acad Sci (2000) 906:8–12. doi:10.1111/j.1749-6632.2000.tb06581.x
28. Van der Vaart M, Pretorius PJ. Circulating DNA. its origin and fluctuation. Ann N Y Acad Sci (2008) 1137:18–26. doi:10.1196/annals.1448.022.
29. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer (2011) 11(6):426–37. doi:10.1038/nrc3066.
30. Huang WL, Wei F, Wong DT, Lin CC, Su WC. The emergent landscape of detecting EGFR mutations using circulating tumor DNA in lung cancer. Biomed Res Int (2015) 2015:340732. doi:10.1155/2015/340732.
31. Fliss MS, Usadel H ,Caballero OL, Wu L, Buta MR, Eleff SM, Jen J and Sidransky D. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science. 2000;287:2017-2019.
32. Gao K, Zhou H, Zhang L,Lee JW, Zhou Q Hu S, Wolinsky LE, Farrell J, Eibl G, Wong DT. Systemic Disease- induced Salivary Biomarker Profiles in Mouse Models of Melanoma and Non Small Cell Lung Cancer. Plos ONE. 2009;4(6):e5875.
33. R. K. Soong, G. D. Bachand, H. P. Neves, A. G. Olkhovets, H.G. Craighead, and C. D. Montemagno, "Powering an inorganic nanodevice with a biomolecular motor," Science, vol. 290, no. 5496, pp. 1555–1558, 2000.
34. A. E. Herr, A. V. Hatch, W. V. Giannobile et al., "Integrated microfluidic platform for oral diagnostics," Annals of the NewYork Academy of Sciences, vol. 1098, pp. 362–374, 2007.
35. R. Kathariya and A. R. Pradeep, "Salivary proteomic biomarkers for oral diseases: a review of literature," Archives of Oral Sciences & Research, vol. 1, pp. 43–49, 2010. R. Kathariya andA. R. Pradeep, "Salivary proteomic biomarkers for oral diseases: a review of literature," Archives of Oral Sciences & Research, vol. 1, pp. 43–49, 2010.

How to cite this article : Jaiswal S., Jahan S., Sharma P.K., Srivastava R.K., Diagnosis in a Spit : A Short Review, Era's Journal of Medical Research.2017;4(1):84-87.