Journal of Academy of Dental Education, 25–29; DOI: 10.18311/jade/2017/20170

ISSN (Print): 2348-1595
ISSN (Online) : 2348-2621

Saliva as Biomarkers

Ooi Yin Ai[1*], Priyanka P.[1] and Sabitha Gokulraj[2]

[1]Intern, Department of Oral Pathology, Vinayaka Mission's Sankarachariyar Dental College , NH – 47, Sankari main road, Ariyanoor , Salem – 636308, Tamil Nadu, India; principal.vmsdc@vmu.edu.in, indiabernice@gmail.com
[2]Senior Lecturer, Department of Oral Medicine and Radiology, Vinayaka Mission's Sankarachariyar Dental College, NH – 47, Sankari main road, Ariyanoor , Salem – 636308, Tamil Nadu, India

Abstract

Saliva is a unique diagnostic fluid that helps in diagnosis of wide range of both systemic and oral diseases. Recent advancement in the field of diagnostics is really helping our health professionals to reach their goal. One of the major advancements in the field of diagnosis is detection of various diseases with the help of saliva. The various biomarkers present in saliva not only help in diagnosis of oral diseases but also various systemic conditions. This review article mainly discusses about the type of biomarkers and the role of saliva in diagnosis of certain diseases and conditions. Therefore, saliva is the promising fluid of the future.

Keywords: Diagnostic Fluid, Point of Care Device, Salivary Biomarkers, Saliva

Date of Receipt: March 2018 Date of Submission: April 2018 Date of Acceptance: May 2018

1. Introduction

Saliva is a unique diagnostic fluid that helps in diagnosis of wide range of both systemic and oral diseases[1]. Saliva plays a major role in diagnosis with the help of biomarkers present in it. Many diseases which were earlier a threat to the current society such as cardiovascular disease, cancer, AIDS, etc can be diagnosed by using saliva. Collection of saliva is undemanding, rapid, non invasive, and it more easier to handle and store, as it does not have the property of clotting[2] and these properties increase the role of saliva in current diagnostic methods.

2. Constituents of Saliva

Saliva is usually the fluid collected from oral cavity and it is mainly secreted by the major and minor salivary glands. The major salivary glands are parotid, sublingual and submandibular glands. Numerous minor glands also contribute to the volume of saliva secreted. Normal saliva is mainly composed of glandular secretion, cervicular fluid, exfoliated epithelial cells, oral bacteria, Immunoglobulin (IgA), digestive enzymes like amylase, zymogen granules and proteases[3,4]. There are also certain biomarkers present which are specific for certain diseases. These biomarkers increase the role of saliva in the field of diagnostics.

3. Biomarkers

Biomarkers refers to a quantifiable biological parameter that is measured and evaluated as an indicator of normal biological, pathogenic, or pharmacologic responses to a therapeutic intervention, according to the National Institutes of Health (NIH). Biomakers can

Address for correspondence:

be classified as: Genomic biomarkers (analysis from DNA), Transcriptomic biomarkers (analysis from RNA), Proteomic or Protein biomarkers (analysis from protein profile), Metabolomic biomarkers (as inter mediates and products of metabolism)

4. Advantages of Saliva

Saliva and other oral fluids are well suited in the diagnostics as they can be collected more easily as they are less susceptible to adulteration[4]. The collection of saliva is safer compared to serum collection, as the risk of transmission of disease can be minimized. This is mostly helpful in cases like viral and bacterial infection. The collection is simpler in obese individuals and children compared to serum[3]. It is also cost effective, does not require much skill and is also non time consuming.

5. Indication of Saliva in Diagnosis

Using microfluidic and Microelectro-Mechanical Systems (MEMS) technologies diagnostic targets such as proteins, metabolites and other small molecules, nucleic acids, human cells, microbes/pathogens and drugs in saliva for various disease would have a significant impact on the future development of salivary diagnostics[5,6]. The below tables shows the different biomarkers present in saliva which helps us to diagnose various diseases.

5.1 Periodontal Diseases[8,9]

Table 1. Inflammatory biomakers for periodontal diseases

Inflammatory Biomakers for Periodontal Diseases	
Inflammatory markers for periodontal diseases	**Description**
a. Beta-glucuronidase-marker of neutrophil influx	At elevated levels it's a risk factor for periodontal attactement loss.
b. c-reactive protein (CRP)	An acute phase reactant that serves as a systemic marker of inflammation; increased levels of CRP are associated with periodontitis
c. Interleukin (IL)-1	A pro-inflammatory cytokine that stimulates the production of adhesion molecules and other mediators, which amplify the inflammatory response that occurs in periodontal diseases
d. Matrix metalloproteinases (MMP)-8	A key enzyme involved in extracellular collagen matrix degradation, derived predominately from polymorphonuclear leukocytes during acute stages of periodontal diseases.
e. Nitric oxide	Oral mucosal disease

5.2 Hormones[8,9]

Table 2. Hormones as biomarkers present in saliva

Biomarkers	Hormones
Alpha amylase Chromogranin A	Stress hormones
Testosterone	Sex hormones
Insulin	Diabetes mellitus

5.3 Autoimmune Diseases[8,9]

Table 3. Biomarkers present in Sjogren's syndrome

Rapid concentrations of NaCl, Ig A, Ig G, lactoferrin, inflammatory mediators such as PGE2, TXB2, Interleukin-6	Sjogren's syndrome

5.4 Cardiovascular Diseases[10]

Cardiovascular diseases refers to group of clinical condition such as ST-elevated myocardiac infarction, non ST-elevated Myocardiac Infarction, unstable angina, etc. the salivary biomarkers which helps us to diagnose cardiovascular diseases are C Reactive Protein (CRP), Myoglobin (MYO), Creatinine Kinase Myocardial Band

(CK-MB), Cardiac Troponins (cTn) and myeloperoxidase. Within 48 hours of onset of acute myocardiac infarction the levels of salivary MYO, myeperoxidase will be increased to a higher level. But, the level of CD40 ligand will be much lesser compare to normal condition. All these biomarkers along with other diagnosing method such as ECG, will add on to the efficiency and effectiveness of diagnosis.

5.5 Oncology[8–10]

Saliva is used as a diagnostic medium in early detection of cancer mainly oral squamous cell carcinoma, breast cancer, ovarian cancer, salivary gland tumours, prostate adenocarcinoma etc. Detection of P53 tumor suppressor protein in saliva/serum indicates early stage of oral squamous cell carcinoma. In cases of breast cancer, CA 15- 3 is the tumor marker which is found in saliva and this helps in monitoring advanced and metastatic cases. Fibroblast Growth Factor 2 (FGF2) and Fibroblast Growth Factor Receptor 1 (FGFR1) concentrations are elevated in saliva in patient with salivary gland tumours. The elevated levels of Prostate Specific Antigen (PSA) in saliva along with the serum PSA level plays as an important biomarker in detection of prostate adenocarcinoma.

5.6 Cystic Fibrosis[8,9]

In cases of cyctic fibrosis there will be increased in level of calcium and phosphate, urea and uric acids and abnormally elevated levels of PGE2.

5.7 Diabetes Mellitus[8,9]

India is the country with the higher rate of diabetes mellitus in 2000 followed by China and united state[7]. This condition can be easily detected by the flow rate of saliva and higher levels of Ig A, salivary peroxidase, glucose content, potassium, saliva total protein and amylase.

5.8 Renal Diseases

The salivary biomarkers which are usually associated with renal diseases are cortisol, nitrite, uric acid, sodium, chloride, ph, amylase and lactoferrin[11]. These biomarkers are usually associated with the end stage of renal diseases[12,13]. The salivary phosphate levels are higher in patients undergoing hemodialysis and chronic renal failure as compared to normal healthy individuals[11]. The salivary phosphate levels plays as a better tool in diagnosis of chronic renal failure and hemodialys is than serum phosphate level.

5.9 Viral Infection[8,9]

The potential usefulness of saliva in diagnosing diseases can be mainly seen in detection of Human Immunodeficiency Virus (HIV). The present of Ig A antibodies in saliva is seen in cases of HIV infection. There are also confirmatory tests like western blot technique/ polymerase chain reaction which is usually done using saliva for detection of p24 antigens and antibodies which is present against both HIV 1 and HIV 2. Furthermore, presence of Ig M antibodies in saliva indicates of acute hepatitis A and B infection.

5.10 Bacterial Infections[8,9]

Monitoring the presence of antibodies and microorganisms found in saliva and oral cavity

5.11 Drugs Monitoring[8,9]

Table 4. Biomarkers as drugs monitoring

Drugs	Conditions
Theophylline	anti asthmatics
Carbamazepine, Diazepam, Ethosuximide,Lamotrigine, Trpiramate, Phenytoin, Primidone, Valproic acid	Anti epileptics
Digoxin, Metoprolol, Procainamide	anti arrhythmic or anti hypetensives
Clarithromycin, Gentamycin, Isoniazid, Ofloxacin, Sulfanilamide	anti microbials
Acetaminophen, Antipyrine,Paracetamol	Antipyretics
cisplastin methotrexate texol, Topotecan	Antineoplastic
Quinine	Anti viral
lithium, amitriptyline	Lithium psychotropic
nicotine ethanol caffeine tolbutamide quinine	Miscellaneous
amphetamine barbiturate benzodiazepines opoids cocaine phencyclidine	illicit drugs

5.12 About Tobacco[14]

The first fluid to encounter with the cigarette smoke in human body is saliva. There are certain biomarkers in saliva which helps to confirm the absorption of specific smoke. One of the major biochemical tests for detecting the prevalence of tobacco consumption includes estimation of level of thiocynate (SCN) in saliva. Thiocyanate is a detoxification product of reaction between cyanide and thiosulfate in liver. So, high level of thiocynate in saliva helps as an indicator of tobacco exposure.

5.13 Sialochemistry Analysis[8,9]

Lead, cadmium, mercury can be detected in early monitoring of an exposure in saliva in cases of metal toxicity.

6. New Technologies

6.1 Point of Care Device (POC)

According to Kost, Gerald J (2002), point of care testing or bedside testing is medical diagnostic testing at or near the point of care that is at the time and place of patient care[15]. It's for current and future use in primary care which is a simple medical test that can perform at the chair side.

Point of care tests systems are easy to use membrane based test strips. It is enclosed by a plastic cassette. Point of care test requires whole blood, urine or saliva to perform and interpret by general physician within minutes. Saliva can be used as a diagnostic tool because it is optimal biological fluid which presents various biomarkers. The major advantages are safe, non-invasive, and simple and can collect repeatedly with minimum discomfort to patient[16].

Various type of biomarkers have been already detected and identified in saliva can be used as clinical parameters. Diagnostic target include: Nucleic acid, proteins, metabolite and small molecules[16,17]. Commonly used devices are Optical detection and electrochemical detection. POC device with optical detection method include absorbance colorimetry, chemiluminescences, fluorescences, surface-enhanced RAMAN scattering spectroscopy and surface plasma resonance[17,18]. Electrochemical detection method include amperometric, potentiometric and impedicmetric measurements[17]. In certain POC devices such as VerOFY device about 100-300 nL of saliva is placed into the salivary collection module, and then delivered into nano-biochip or microbead array-micro pits within a silicon wafer are populated with a variety of chemically sensitized bead micro reactors.

The specifically designed portable scanning module LIAM (Lite Image Analysis Module) is used to quantify the results from VerOFy Rapid, Oral fluid test. This technique also offers the ability to transfer files to other devices.

7. Conclusion

The different biomarkers which are present in the saliva thus help the health professional to diagnose not only oral diseases but also systemic diseases and conditions at an earlier stage. Rather, collection of saliva is simpler, non-invasive, low cost method which gives more rapid and accurate results as compared to serum. However, further standardized collection devices and assay are still required for sampling and testing of saliva. And, if these deficiencies are met, salivary tests can definitely supersede other common serological tests.

8. Reference

1. Greabu M, Calenic B. Saliva: A diagnosis fluid for oral and general diseases. Organic Chem Cur Res. 2014; 04:131.
2. Wang A, Wang CP, Tu M, Wong DTW. Oral biofluid biomarker research: Current status and emerging fronties. Diagnostics (Basel). 2016 Dec; 6(4):45. Crossref. PMid:27999326 PMCid:PMC5192520
3. Tamashiro H, Constantine NT. Serological diagnosis of HIV infection using oral fluid samples. Bulletin of the World Health Organization. 1994; 72(1):135–43. PMid:8131250 PMCid:PMC2486497
4. Henson B, Zentz R, Wong DT. A primer on salivary diagnostics. ADA; 2005 Mar.
5. Gubala V, Harris LF, Ricco AJ, Tan MX, Williams DE. Point of care diagnostics: Status and future. Anal Chem. 2012 Jan 17; 84(2):487–515. Crossref. PMid:22221172
6. Gau V, Wong D. Oral fluid nanosensor test (OFNASET) with advanced electrochemical-based molecular analysis platform. Annals of the New York Academy of Sciences. 2007 Apr; 1098:401–10. Crossref. PMid:17435145
7. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. The Australasian Medical Journal (AMJ). 2014; 7(1):45–8. Crossref.

8. Silberring J, Ciborowski P. Biomarker discovery and clinical proteomics. Trends Anal Chem Trac. 2010; 29:128. Crossref. PMid:20174458 PMCid:PMC2822390
9. Millar CS, Foley JD, Bailey Al, et al. Current developments in salivary diagnostics. Biomarkers Med. 2010; 4:171–89. Crossref.
10. Malathi N, Mythili S, Vasathi HR. Salivary diagnostics: A brief review. ISM Dentistry. 2014; 2014:8. Crossref.
11. Daniel Malamud and Issac Radriguez-chavez. Saliva as a diagnostic fluid. HHS public access author manuscript. Dent Clin North Am. 2011 Jan; 55(1):159–78. Crossref. PMid:21094724 PMCid:PMC3011946
12. Walt DR, Blicharz TM, Hayman RB, et al. Microsensor arrays of saliva diagnostic. Ann NY Acad Sci. 2007; 1098:389–400. Crossref. PMid:17435144
13. Arregger Al, Ardosoem, Tumilasci O, et al. Diagnostic value of salivary cortisol in end stage renal disease. Setroids. 2008; 73(1):77–82. Crossref. PMid:17945323
14. Pullishery F, Panchnal GS, Siddique S. Salivary thiocynate, uric acid and PH as biomarkers of periodontal diseases uses and ion-user-an-in-vitro study. J Clin Diagn Res. 2015 July; 9(7):zc47–zc50. PMid:26393205 PMCid:PMC4551899
15. Kost GJ. Principles and practice of point of care testing. Philadelphia: Lippincott Williams and Wilkins; 2002. p. 3–12.
16. Song, et al. Point of care technologies for molecule diagnostics using a drop of blood. Trends Biotechnology. 2014; 32:132–9. Crossref. PMid:24525172 PMCid:PMC3962833
17. Su W, Gao X, Jiang L, Qin J. Microfluidic platform towards point of care diagnostics in infectious diseases. J Chromatogr A. 2015; 1377:13–26. Crossref. PMid:25544727
18. Gubala V, Harris LF, Ricco AJ, Tan MX, Williams DE. Point of care diagnostics: Status and future. And Chem. 2012; 84:487–515.Crossref.