Saraswathi K. Gopal et al /J. Pharm. Sci. & Res. Vol. 13(11), 2021, 677-680

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Salivary Biomarkers – A Modern Approach to Diagnosis

Dr. Saraswathi K. Gopal[1], Dr. Preethi Ravi [2], Dr. Srividhya[3]
[1]Professor and Head of the Department, [2]PG Student, [3]Associate Professor,
Department of Oral Medicine and Radiology,
Meenakshi Ammal Dental College, Chennai-600095

Abstract

Saliva is a biofluid that has immense diagnostic potential. Saliva in diagnostics is a promising approach as collecting saliva is easy and non-invasive. The biomarkers are crucial to detect underlying pathologies for early diagnosis and to induce quality of life in patients. The search for biomarkers has drawn attention in medical sciences. The salivary biomarkers specifically for early cancer detection such as cancers occurring in the oral cavity and oropharynx have been in implementation for the past two decades. This review gives an update on the contribution of salivary biomarkers to the diagnosis and prognosis of diseases of the oral cavity, including oral cancer, and other systemic diseases.

Keywords: Salivary biomarkers; Saliva, Diagnosis, Oral and systemic diseases, Oral cancer

INTRODUCTION

The oral cavity consists of multiple structures working in harmony. Saliva is an integral part of the oral cavity which is a secretory product of major and minor salivary glands[1]. Salivary analysis is a non-invasive procedure, which is an attractive alternative option for the diagnosis and prognosis of oral diseases. Metabolites, electrolytes are passively filtered into the saliva which is in harmony with the systemic and environmental conditions of an individual which are involved in the assessment of biomarkers. Saliva is beneficial for laboratory diagnosis, prognosis, and management of patients with both oral and systemic diseases as it is easily collected and stored and ideal for early detection of disease because it contains specific soluble biological markers.

SALIVA

Saliva is a clear, slightly acidic mucoserous exocrine secretion It is a complex mixture of fluid from major and minor salivary glands along with mucosal transudate and various other metabolites. It is slightly acidic (pH=6.0–7.0) in nature. The normal salivary secretion is 1 to 1.5 liter per day for an adult.[1]. The mode of entry of various metabolites from the blood into the saliva occurs intracellularly by passive diffusion and active transport. There can also be other routes (paracellular) by extracellular ultrafiltration [2,3] Saliva, known as the 'mirror of the body', which is a perfect medium to be applied for clinical diagnostics.[4]

ADVANTAGES OF SALIVA AS A DIAGNOSTIC TOOL [5]

- Non-invasive
- Easy to store & transport
- Cost-effective
- Saliva sample can be collected multiple times
- Used in clinically challenging situations, such as from children, physically challenged, or anxious patients in whom blood sampling could be difficult to perform.

Saliva can be collected in various forms –
a) Unstimulated whole saliva,
b) Stimulated whole saliva
Unstimulated saliva is the secretion produced in the absence of exogenous stimuli. Stimulated saliva is secreted as a response to either masticatory or gustatory stimulations[6] In salivary diagnostics, unstimulated saliva is often preferred to the stimulated whole saliva because stimulated whole saliva contains only a diluted concentration of biomarkers, that may be difficult to detect [6].

SALIVA COLLECTION METHODS

There are various methods to collect the whole saliva such as draining, spitting, suction, and absorbent methods [1]. Shannon (2008) has compared flow rates in a group of subjects in different body positions i.e. standing position, lying position, and sitting position, and concluded that it is ideal to collect saliva, while the subject is sitting upright with the head slightly tilted forward and the eyes open.[6]

- **DRAINING METHOD /SPITTING METHOD**: The patient allows saliva to accumulate in the mouth and then expectorate into a pre-weighed test tube usually every 60 seconds for 5-15 minutes
- **SUCTION METHOD:** Aspirator or saliva ejector is used to collect saliva from the mouth into a test tube.
- **ABSORBENT METHOD**: A preweighed gauze is placed in the patient mouth for a predetermined amount of time, following which the gauze is weighed again, and the volume of saliva is determined.

BIOMARKERS [7]

According to The National Institutes of Health Biomarkers Definitions Working Group, a biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." According to World Health Organization (WHO), a biomarker is "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease"

CHARACTERISTICS OF AN IDEAL BIOMARKER [8]

- Should be Easy and inexpensive to measure in readily available body fluids
- Should change as the current status of the disease changes over time
- Should predict the recurrence of the disease, before they are clinically detectable.

BIOMARKERS IN BLOOD AND SALIVA [3]

Blood, which contains molecular components, such as enzymes, hormones, antibodies, and growth factors, is most commonly used to detect biomarkers. But its main drawback is the procedure of collecting and analyzing blood, which is expensive and physically intrusive. Hence saliva, an accessible fluid with a non-invasive extraction method, which overcomes these drawbacks, is used as a medium for biomarker assessment. Various biomarkers detected in the blood serum are also found in saliva, but their levels vary i.e. levels in serum are several-fold higher than those found in saliva. But various biomarkers in saliva are found to be more sensitive to certain diseases when compared to serum. Example: Carcinoembryonic antigen (CEA) is elevated in serum and saliva in oral cancer. But salivary CEA is a more sensitive indicator. Hence, saliva is considered as a potential alternative to blood.

BIOMARKERS IN EARLY CANCER DETECTION

Oral cancers in the advanced stage are detected by clinical examination of the oral cavity[9]. But early-stage oral carcinomas are frequently not diagnosed in time, because even the microscopic level for progressive cancer can be too late for successful intervention. Hence the biomarkers play an important role in early cancer detection by reducing the lethal outcome of this dangerous disease.

Potential salivary biomarkers for oral cancer detection [9,14,15,16]

Table 1:

MOLECULES	BIOMARKERS	
Peptide	Defensin-1	Upregulated
Proteins	P53 autoantibody IL-1 IL-6 IL-8 IL-1β TNF-α Cancer antigen 125 (CA125) Carcinoembryonic antigen (CEA)	Upregulated
	Amylase	Downregulated
Metabolites	Lactic acid Cadaverine Alanine Hypoxanthine Methionine	Upregulated
Oxidative stress-related molecules	• Reactive nitrogen species – nitric oxide (NO), nitrites (NO2), nitrates (NO3) • Glutathione S-transferase • Superoxide dismutase • 8-hydroxy-2-deoxyguanosine • Malondialdehyde	Upregulated
DNA	P53 gene codon 63	Mutation
mRNA	IL8	Increased

IL-6, IL-8, IL-1β, basic fibroblast growth factor, which is involved in inflammation, are important biomarkers in cancer detection and are found to be upregulated in oral squamous cell carcinoma [9.] Salivary peptides such as CD59, defensin-1, catalase proteins; and carcinoembryonic antigen (CEA) are sensitive biomarkers in the detection of oral cancer because of its high sensitivity and specificity for oral cancer diagnosis. The sensitivity and specificity of these salivary peptides are approximating 90% and 80% respectively and for carcinoembryonic antigen (CEA), which are inhibitors of apoptosis, the sensitivity and specificity values are 76.4% and 80.4%, respectively.[11] In saliva, mutated salivary DNA at the p53 gene was found in 62.5% of oral cancer patients [10]. Salivary miRNA is used as a biomarker to assess the progression of oral potentially malignant disorders (OPMD) to OSCC [12]. There is increased expression of miR-708, miR-10b, miR-19a, miR-30e, miR-26a, and miR-660 and decreased expression of miR-99, miR-15a, miR-197, miR-145, and miR-150 in saliva, which represents the malignant transformation of oral potentially malignant disorders. [13,14] Stress alters the salivary composition in OSCC patients. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels are markedly increased in OSCC patients. [14]

Salivary Biomarkers in Oral Diseases:

Salivary biomarkers can be useful for the diagnosis, monitoring, and prognosis of various oral diseases such as oral leukoplakia for its malignant transformation potential, oral lichen planus, Oral Submucous Fibrosis**,** Medication-Related Osteonecrosis of the Jaw, Sjögren's syndrome, Pemphigus, and Pemphigoid.

Oral Lichen Planus [5,18,19,20,21]

Biomarker	Level in saliva	Clinical implication
Cortisol	Increased	Diagnosis
Nitric Oxide, IL6	Increased	Prognosis
IL1α IL1β IL8	Increased	Immune and inflammatory response modulator

Oral Leukoplakia [5,22,23,24,25]

Biomarker	Salivary Levels	Clinical Relevance
TNF- α TGFβ	Increased	Detection of malignant transformation of leukoplakia into OSCC
IL6 IL8	Increased	Prognosis

Oral Submucous Fibrosis [26,27]

Biomarkers	Level in saliva	Clinical implications
IgA	Increased	Early detection of the transformation process of OSMF to oral carcinoma.
IgG	Increased	Diagnosis
Total serum protein	Decreased	Prognosis

Pemphigus and Pemphigoid [28,29]

Oral Pathology	Biomarker	Level in saliva	Clinical implications
Pemphigus	Desmoglein1 and Desmoglein 3	Increased	Diagnosis
Pemphigoid	IgG and IgA	Increased	Diagnosis

Medication-Related Osteonecrosis of the Jaw [5, 30,31,32]

Biomarker	Level in saliva	Clinical implications
IL1α, IL1β, IL1RA, IL6, MMP9	Increased	Diagnosis

Primary Sjögren Syndrome [5,33,34]

Biomarker	Salivary Levels	Clinical implications
IL12, CD44, SP1, PSP, CA6	Increased	Diagnostic and prognostic value

SALIVARY BIOMARKERS IN SYSTEMIC CONDITIONS [35,36,37,38,39,40]

Diseases	Salivary biomarkers	Salivary Levels
Diabetes mellitus	• Glucose	Increased
Renal condition	• Cortisol • Nitrite • Alpha-amylase • Lactoferrin • Creatinine	Increased
Bone turnover markers	• Osteocalcin • Hepatocyte growth factor • Interleukin-1-beta • Alkaline phosphatase	Increased
Cardiovascular diseases	• C-reactive protein • Myoglobin • Creatinine kinase myocardial band • Cardiac troponins • Myeloperoxidase • Tumor necrosis factor α • Matrix metalloproteinase–9 • Lysozyme	Increased
Psychological Stress	• Cortisol • α-amylase • Proinflammatory cytokines (IL-6, IL-1b, TNF- α) • Catecholamines (dopamine, norepinephrine, and epinephrine)	Increased

AUTOIMMUNE DISEASES [35,41,42,44,45]

Autoimmune diseases	Salivary biomarkers	Salivary Levels
Multiple sclerosis	IgA levels	Decreased
Sjogren's syndrome	Kallikrein Lactoferrin Lysozyme C Amylase Carbonic anhydrase	Increased Increased Increased Decreased Decreased
Sarcoidosis	Interleukin-2 Interleukin-6	Increased Increased
Rheumatoid arthritis	TNF- α IL1β	Increased Increased

CONCLUSION

Saliva, the safest, non-invasive, cost-effective biofluid consists of numerous biomarkers associated with various diseases. Salivary levels of various biomarkers are known to change in the presence of various diseases of the oral cavity and are found to be useful for their diagnosis and prognosis. Their evaluation in saliva offers clinicians a valuable procedure as a complement to clinical findings. Hence Saliva is a 'boon' in the field of diagnostics.

REFERENCES:

1. Burket's Oral Medicine. Publication Year: 2014. Edition: 12th
2. Podzimek S, Vondrackova L, Duskova J, Janatova T, Broukal Z, Salivary markers for periodontal and general diseases. Dis Markers 2: 1-6, 2016.
3. Janice M. Yoshizawa, a Christopher A. Schafer, a Jason J. Schafer,b James J. Farrell,c Bruce J. Paster,d,e David T. W. Wonga, Salivary Biomarkers: Toward Future Clinical and Diagnostic Utilities., Clinical Microbiology Reviews p. 781–791, October 2013
4. T.K Fábián, P Fejérdy, P Csermely, Salivary Genomics, Transcriptomics, and Proteomics: The Emerging Concept of the Oral Ecosystem and their Use in the Early Diagnosis of Cancer and other Diseases, Curr Genomics. 2008 Mar; 9(1): 11–21.
5. Lucía Melguizo-Rodríguez, Victor J. Costela-Ruiz,Francisco Javier Manzano-Moreno, Concepción Ruiz, Rebeca Illescas-Montes, Salivary Biomarkers and Their Application in the Diagnosis and Monitoring of the Most Common Oral Pathologies, Int J Mol Sci. 2020 Jul; 21(14): 5173.
6. Navazesh M, Methods for collecting saliva, Annals of the New York Academy of Sciences. 1993 Sep 1;694(1):72-7.
7. A. Zhang, H. Sun, P. Wang, X. Wang, Salivary proteomics in biomedical research, Clin. Chim. Acta 415 (2013) 261e265.
8. Shikha Saxena, Bharat Sankhla, Krishna Sireesha Sundaragiri, and Akshay Bhargava, A Review of Salivary Biomarker: A Tool for Early Oral Cancer Diagnosis, Adv Biomed Res. 2017; 6: 90.
9. Yi-Shing Lisa Cheng, Terry Rees, and John Wright, A review of research on salivary biomarkers for oral cancer detection, Clin Transl Med. 2014
10. Zohaib Khurshid, Muhammad S. Zafarx, Rabia S. Khanjj Shariq Najeeb, Paul D. Slowey, and Ihtesham U. Rehmanjj, Role of Salivary Biomarkers in Oral Cancer Detection, Advances in Clinical Chemistry, February 2020
11. J Kaur1 & R Jacobs & Y Huang & N Salvo & C Politis, Salivary biomarkers for oral cancer and pre-cancer screening: a review, Clinical Oral Investigations, Jan 2018
12. Y, St John MA, Zhou X, Kim Y, Sinha U, Jordan RC, Eisele D, Abemayor E, Elashoff D, Park NH, Wong DT, Salivary transcriptome diagnostics for oral cancer detection.Li, Clin Cancer Res. 2004 Dec 15; 10(24):8442-50.
13. Poornima G, Mahesh Kumar TS. Genomic Alphabets of Saliva as a Biomarker in Oral Cancer. J Indian Acad Oral Med Radiol 2017;29:300-5

14. Yang, Y., Li, Y. X., Yang, X., Jiang, L., and Zhou, Z. (2013). Progress risk assessment of oral premalignant lesions with saliva miRNA analysis, *BMC Cancer* 13:129.
15. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, Wong DT: Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection, Clin Cancer Res 2009, 15:5473-5477.
16. Snehashish Ghosh, Preeti Singh, K. Shwetha Nambiar, Vanishri C. Haragannavar, Dominic Augustine, S. V. Sowmya, Roopa S. Rao, Role of salivary biomarkers in the diagnosis of oral cancer: Mini review, Journal of Medicine, Radiology, Pathology & Surgery (2018), 5, 7–10
17. Daniel Malamud, Isaac R. Rodriguez-Chavez, Saliva as a Diagnostic Fluid, Dent Clin North Am. 2011 January; 55(1): 159–178
18. Humberto J.S.M., Pavanin J.V., Da Rocha M.J.A., Motta A.C.F, Cytokines, cortisol, and nitric oxide as salivary biomarkers in oral lichen planus: A systematic review. Braz. Oral Res. 2018;32 DOI: 10.1590/1807-3107bor-2018.vol32.0082
19. Mozaffari H.R., Ramezani M., Mahmoudiahmadabadi M., Omidpanah N., Sadeghi M., Salivary and serum levels of tumor necrosis factor-alpha in oral lichen planus: A systematic review and meta-analysis study, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 2017;124:e183–e189.
20. VR Rekha, S Sunil, R Rathy, Evaluation of oxidative stress markers in oral lichen planus, J Oral Maxillofac Pathol. 2017 Sep-Dec; 21(3): 387–393.
21. Y. Hassona, C. Scully, Salivary changes in oral mucosal diseases, *Periodontology 2000*, vol. 70, no. 1, pp. 111–127, 2016.
22. Deepthi G,1, S R K Nandan, Pavan G Kulkarni, Salivary Tumour Necrosis Factor-α as a Biomarker in Oral Leukoplakia and Oral Squamous Cell Carcinoma, Asian Pac J Cancer Prev. 2019; 20(7): 2087–2093.
23. Sharma M, Bairy I, Pai K, Satyamoorthy K, Prasad S, Berkovitz B, et al. Salivary IL-6 levels in oral leukoplakia with dysplasia and its clinical relevance to tobacco habits and periodontitis. Clin Oral Investig 2011;15:705-14
24. Punyani SR, Sathawane RS. Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma. Clin Oral Investig 2013;17:517-24.
25. Brailo V, Vucićević-Boras V, Cekić-Arambasin A, Alajbeg IZ, Milenović A, Lukac J. The significance of salivary interleukin 6 and tumor necrosis factor-alpha in patients with oral leukoplakia. Oral Oncol 2006;42:370-3.
26. Dr. S. Priyanka, K Saraswathi Gopal, Dr. A. Vignesswary, Correlation of serum, salivary IgA with serum, salivary total proteins in oral submucous fibrosis patients"- a study, World Journal of Pharmacy and Pharmaceutical Sciences, 2016
27. Kalpana A Patidar 1, Rajkumar N Parwani, Sangeeta Panjab Wanjari, Correlation of salivary and serum IgG, IgA levels with total protein in oral submucous fibrosis, J Oral Sci,2011 Mar;53(1):97-102.
28. Z. Hallaji, H. Mortazavi, V. Lajevardi et al., "Serum and salivary desmoglein 1 and 3 enzyme-linked immunosorbent assay in pemphigus vulgaris: correlation with phenotype and severity," Journal of the European Academy of Dermatology and Venereology, vol. 24, no. 3, pp. 275–280, 2010.
29. S. Ali, C. Kelly, S. J. Challacombe et al., Salivary IgA and IgG antibodies to bullous pemphigoid 180 noncollagenous domain 16a as diagnostic biomarkers in mucous membrane pemphigoid, The British Journal of Dermatology, vol. 174, no. 5, pp. 1022–1029, 2016.
30. Bagan J., Sheth C.C., Soria J.M., Margaix M., Bagan L. Bisphosphonates-related osteonecrosis of the jaws: A preliminary study of salivary interleukins. J. Oral Pathol. Med. 2012;42:405–408.
31. Bagan J., Sáez G., Tormos M., Hens E., Terol M., Bagan L., Diaz-Fernandez J., Lluch A., Camps C. Interleukin-6 concentration changes in plasma and saliva in bisphosphonate-related osteonecrosis of the jaws. Oral Dis. 2013;20:446–452.
32. Thumbigere-Math V., Michalowicz B.S., De Jong E.P., Griffin T.J., Basi D.L., Hughes P.J., Tsai M.L., Swenson K.K., Rockwell L., Gopalakrishnan R. Salivary proteomics in bisphosphonate-related osteonecrosis of the jaw. Oral Dis. 2013;21:46–56.
33. Ben-Chetrit, E.; Fischel, R.; Rubinow, A., Anti-SSA/Ro and anti-SSB/La antibodies in serum and saliva of patients with Sjogren's syndrome. Clin Rheumatol. 1993, 12, 471–474.
34. Ching, K.H.; Burbelo, P.D.; Gonzalez-Begne, M.; Roberts, M.E.P.; Coca, A.; Sanz, I.; Iadarola, M.J. Salivary anti-Ro60 and anti-Ro52 antibody profiles to diagnose Sjogren's Syndrome. J. Dent. Res. 2011, 90, 445–449.
35. Marco Meleti,1 Diana Cassi, Paolo Vescovi, Giacomo Setti, Thelma A. Pertinhez, and Margherita Eleonora Pezzic, Salivary biomarkers for diagnosis of systemic diseases and malignant tumors. A systematic review, Med Oral Patol Oral Cir Bucal. 2020 March.
36. Bagan J., Sáez G., Tormos M., Hens E., Terol M., Bagan L., Diaz-Fernandez J., Lluch A., Camps C. Interleukin-6 concentration changes in plasma and saliva in bisphosphonate-related osteonecrosis of the jaws. Oral Dis. 2013;20:446–452
37. Patrícia Batista, Salivary Biomarkers in Psychological Stress Diagnosis, ARC Journal of Pharmaceutical Sciences (AJPS), 2017
38. Marrelli M, Gentile S, Palmieri F, Paduano F, Tatullo M. Correlation between Surgeon's, experience, surgery complexity, and the alteration of stress-related physiological parameters. PLoS One. 2014;9:e112444.
39. P Sai Archana, K Saraswathi Gopal, B G Harsha Vardhan, P Mahesh Kumar, Saliva as a Non-invasive Tool in Evaluation of Type 2 Diabetes Mellitus, International Journal of Scientific Study | April 2016, Vol 4, Issue 1
40. Prathibha KM, Johnson P, Ganesh M, Subhashini AS. Evaluation of salivary profile among adult Type 2 diabetes mellitus patients in South India. J Clin Diagn Res 2013;7:1592-5.
41. Eklund KK, Leirisalo-Repo M, Ranta P, Maki T, Kautiainen H, Hannonen P, Korpela M, Hakala M, Jarvinen P, Mottonen T. Serum IL-1beta levels are associated with the presence of erosions in recent onset rheumatoid arthritis. Clin Exp Rheumatol. 2007;25:684–689
42. Jeffrey Mirrielees, D.M.D., Leslie J. Crofford, M.D.,† Yushun Lin, M.S. Richard J. Kryscio, Ph.D., Dolphus R. Dawson, III, D.M.D., M.S., Jeffrey L. Ebersole, Ph.D., and Craig S. Miller, D.M.D., M.S., Rheumatoid Arthritis and Salivary Biomarkers of Periodontal Disease, J Clin Periodontol. 2010 Dec; 37(12): 1068–1074.
43. Alberto Muñoz-Prieto, Eduardo Pons-Fuster, Pia López-Jornet, Salivary Markers in Inflammatory and Autoimmune Diseases, Saliva in Health and Disease pp 177-192|
44. Kang EH, Lee YJ, Hyon JY, Yun PY, Song YW. Salivary cytokine profiles in primary Sjogren's syndrome differ from those in non-Sjogren sicca in terms of TNF-alpha levels and Th1/Th-2 ratios. Clin Exp Rheumatol 2011; 29(6): 970-976
45. Ohyama K, Moriyama M, Hayashida J, Tanaka A, Maehara T, Ieda S, et al. Saliva as a potential tool for diagnosis of dry mouth including Sjögren's syndrome. Oral Dis 2015; 21(2): 224-231. DOI: 10.1111/odi.12252