Original Research Paper

Volume - 10 | Issue - 11 | November - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Radiology

NEW APPROACH FOR THE DIAGNOSIS OF SYSTEMIC DISEASE BASED ON SALIVARY BIOMOLECULES

Dr.Priya Ramani	Professor, Department Of Oral Medicine And Radiology, Thai Moogambigai Dental College &Hospital.
R.Nishanthi*	Junior Resident, Thai Moogambigai Dental College & Hospital. *Corresponding Author
S.Nivas Sundar	Junior Resident, Thai Moogambigai Dental College & Hospital.
R.Pooja	Junior Resident, Thai Moogambigai Dental College & Hospital,

ABSTRACT Human saliva is packed with proteins that helps control the teeming hordes of microbes in our mouth. Whole saliva is a mixture of secretions from major and minor salivary glands, mucosal transdations, gingival crevicular fluid, serum and blood derivatives from oral wounds, nasal secretion, desquamative epithelial cells, microbes and food debris. Currently, Systemic diseases are very challenging to get diagnosed, without more invasive supplementary investigations. In order to overcome this condition, medical researcher found molecular disease biomarkers that can be easily identified and successfully implement a non-invasive and a fast diagnosis. Biomarkers in saliva have been identified for wide variety of diseases and conditions. In the near future, salivary samples are ready to distinguish between bacterial and viral infections and detect recent heart attacks, traumatic brain injury, even lung and gastric diseases etc. Saliva as a diagnostic tool may fulfil the objectives by providing a safe and effective means to evaluate patients and personalize their treatment modalities.

KEYWORDS :

INTRODUCTION:

The human body has a higher quantity of proteins that consist of sort of fluids, such as blood, urine and saliva which is related to several systemic and oral diseases. So, these fluids proved to have found widespread clinical applications in order to diagnose and monitor human health (1). During the path of research, three main limitations have influenced the development and research of specific biomarkers for early disease detection:

- so lack of definitive molecular biomarkers for specific diseases,
- lack of easy and inexpensive sampling method with minimal discomfort, and
- The lack of accurate and easy-to-use platform that can facilitate the early detection (2).

Saliva maybe a complex fluid which contains a far better number of hormones, proteins, antibodies, cytokines, and antimicrobial constituents which can facilitate their associations with a selection of spread of systemic diseases (3). Saliva is a biological fluid capable of detecting certain diseases and would be useful for approaches to prognosis, laboratory or clinical diagnosis, monitoring and management of patients with both oral and systemic diseases. Besides, it is very easily collected and stored for early detection of disease as it contains specific soluble biological markers (biomarkers) (4).

SALIVA CONSTITUENTS :-

The salivary fluid is an exocrine secretion that consists of roughly 99% water, with a spread of electrolytes like sodium, potassium, calcium and phosphate and proteins like enzymes, immunoglobulin, antimicrobial factors, polypeptides and oligopeptides, also traces of albumin, and mucosal glycoprotein of great importance in maintaining a balance of the oral health. Saliva also contains glucose, urea, and ammonia in various quantities that are responsible for several systemic diseases (5).It also contains high concentrations of Na+, Cl–, Ca2+, K+, HCO3, H2PO4 and Mg2+ from the serum. In addition, also contains >700microorganisms that are related to oral and systemic diseases(6). The oral fluid originates predominately from three pairs of major salivary glands (parotid, sublingual, and submandibular) and from numerous minor salivary glands (7). The study on human saliva as characterized 4 major types of salivary proteins: PRPs, cystatins, statherins , and histatins. Saliva contributes to many function (Table-01). The important role of this type of proteins is to maintain the integrity of tooth structures in the oral cavity, especially involved in the demineralization and remineralisation process of the enamel(8).

The significance of Saliva used as a diagnostic fluid: -

Saliva have a distinctive advantage comparing to serum, that can be collected non-invasively and also does not require any special equipment for collection and storage. It is advantageous for people, in whom blood drawing is difficult as in obese, haemophiliacs and patients who are fear of prick. WS are often used for diagnosis of systemic diseases, because it contains serum constituents, for a few diagnostic purposes, also salivary biomarkers proved to be more useful than serum analysis (9).

The advantages for salivary biomarker detections are;

- It should have a real-time diagnostic values
- Safe for health professionals than blood sampling
- should be ease of obtaining multiple samples
- Where collection and screening can be done at home
- It is non invasive, easy and inexpensive
- It has a minimal risk of cross-contamination
- More of economical sampling, shipping and storage
- H. Manipulation is required during diagnostic procedures is less as compared to that of serum
- Commercial availability of screening assay
- Also, saliva does not clot unlike blood (10).

Today, since technology is at some extent are readily detectable employing number of methodologies, proteomics, mass spectrometry, genome wide association studies and other screening technique. Biomarker in saliva has been identified for a wide variety of diseases and conditions (11).

Table-1 Functions Of Saliva:

FUNCTION	EFFECT	ACTIVE CONSTITUENTS
Protection	Clearance Lubrication Thermal/chemical insulation Pellicle formation Tannin binding	Water Mucins, glycoproteins Mucins Protein, glycoproteins, mucins Basic proline-rich proteins, histatins
Buffering	pH maintenance Neutralization of acids	Bicarbonate, phosphate, basic protein, urea, ammonia
Tooth integrity	Enamel maturation, repair	Calcium, phosphate, fluoride, statherin, acidic proline-rich proteins.
Anti-microbial activity	Physical barrier Immune defense Non-immune defense	Mucins Secretory immunoglobulin A Peroxidase, lysozyme, lactoferrin, histatin, mucins, agglutinins, secretory leukocyte protease inhibitor, defensins and cathelicidin-LL37

Tissue repair	Wound healing, epithelial	Growth factors, trefoil proteins, regeneration
Digestion	Bolus formation Starch, triglyceride digestion	Water, mucins Amylase, lipase
Taste	Solution of molecules Maintenance of taste buds	Water and lipocalins Epidermal growth factor and carbonic anhydrase VI

Table 2: Salivary biomarkers in various systemic disease

S.NO	DISEASE	BIOMARKER LEVEL	SOURCE OF BIOMARKERS
1.	AUTO-IMMUNE • Multiple sclerosis	Low lactoferrin, beta2 microglobulin,lysozyme C, cystatin C and low levels of salivary amylase & carbonic anhydrase	Saliva
	• Sarcoidosis	Low Alpha-amylase & kallikrein	Serum and saliva
2.	Bone turnover markers	Analysis of D-PYR, OC concentration, hepatocyte growth factor, interleukin-1 beta, salivary osteonectin, and ALP activity.	Serum and saliva
3.	Dental caries and periodontal disease	Streptococcus mutans and lactobacilli count, aspartate aminotransferase,Alkaline phosphatase.	Saliva
4.	MALIGNANCY • Oral squamous cell carcinoma	MiRNAs and p53 antibiomarkers.	Serum and saliva
5.	Renal diseases	Cortisol, nitrite, uric acid, sodium chloride, pH, alpha-amylase and lactoferrin, salivary phosphate, serum creatinine and saliva glomerular filtration rate	Serum and saliva

DIAGNOSIS OF SYSTEMATIC DISEASES BY SALIVA

There are some characteristic changes that may contribute to the diagnosis and early detection of these diseases, they are (12)

- Hereditary disease
- Autoimmune disease
- Malignancy disease
- Infectious disease
- Hormonal disease

A)HEREDITARY DISEASE :

Cystic fibrosis:

Cystic fibrosis (CF) is a autosomal – recessive disorder which is genetically transmitted to children and young adults. The gene defect is present in chromosome 7 (13). Cystic fibrosis affects the cells that produce mucus, sweat and digestive juices. These secreted fluids are normally thin and slippery. The organs that are mostly affected are the sweat glands, the lungs developing COPD, and the pancreas resulting in pancreatic insufficiency (14).CF in saliva collected from the affected patient shows epidermal growth factor (EGF).It shows a poor biological activity when compared to a healthy patient, showing ECG anomaly (15). It is identified in the diseased patient by detecting the presence of caries that is asymptomatic and cannot be detected by diagnostic tests that is carried out. Due to higher number of mutated genes in CF the identification is very difficult (16).

Coeliac disease:

Coeliac disease is a congenital disorder , It mainly affects the small intestine that involves the gluten malabsorption. It contains elevated level of serum IgA antigliadin (AGA) (17). Because of higher sensitivity and lower specificity is very difficult, so screening of salivary IgA-AGA in serum is not recommended (18).

21-Hydroxylase deficiency:

It is a inherited disorder that results in congenital adrenal hyperplasia. The screening test of this deficiency shows us non-classic 21-hydroxylase deficiency (17-OHP) (19).

B)AUTOIMMUNE DISEASE:-

Sjogrens syndrome:

Sjogren's syndrome (SS) is an autoimmune disease where the cause is unknown etiology. Mainly females are mostly affected. It is mainly associated with keratoconjunctivits sicca and xerostomia showing reduction in lacrimal and salivary secretions. So presence of these two diagnosis leads to primary SS, whereas in secondary SS shows a well defined connective tissue disease. In secondary SS there is presence of rheumatoid arthritis or systemic lupus erythematosus in addition to xerostomia and/or the keratoconjunctivits (20).

Sjogren's syndrome mainly involves the salivary and lacrimal gland and also affects the skins, lungs, liver, kidney and nervous system (21).The elevated chemicals that are present in the saliva of these patients are;

a. Increase in sodium and chloride
b. Elevated levels of IgA, IgG, lactoferrin and albumin
c. Soluble IL-2 receptor is seen.

The diagnosis and early detection of this syndrome is very difficult. It shows a reduced salivary flow which is an important feature to be noted by the dentist (12).

C)MALIGNANCY:-

Breast cancer:-

One of the most common cancers in females is the breast cancer.ATP6AP1 is an ATPase that is expressed in normal tissues like brain marrow, blood, nerves and skin and it is also with several tumours such as head and neck carcinomas, lung tumours, adrenal tumours and various other cancers. ATP6AP1 is an auto antibody that is spontaneously generated in patients and can be detected in the early stages. Thus, it is indicated that ATP6AP1can contribute to early detection of the breast cancer (22).In another study, it was found that levels of vascular endothelial growth factor, epidermal growth factor (EGF) and carcino embryonic antigen in the saliva were found increased in patients with breast cancer (23). The levels of CA15-3 and c-erB-2 were found to be increased in the saliva, in the serum of patients having breast cancer. Based on these studies, salivary biomarkers can be applied for the early diagnosis of breast cancer (24). Certain malignant tumours on early diagnosis and detection are useful in salivary analysis. p53 a tumour suppressor protein is exposed causing DNA damaging through mutation and gene deletion leads to occurrence in development of human cancer (25).

Ovarian cancer

The tumour marker for epithelial cancer is CA 125.When it shows elevated level then the patient is detected to have ovarian cancer. A positive correlation is seen between the saliva and serum level of CA 125 (25).tumour markers are very useful in detecting the malignant disease and additional diagnosis helps to determine and used for diagnostic purpose of malignancy (12).

Prostate cancer

MiR-141 and miR-21 are the two tumour biomarkers; the former is elevated in patients with advanced-stage prostate cancer, whereas the latter is over expressed in the early-stage prostate cancer. It has been demonstrated that the expression of miR-21 and miR-141 in the saliva can be only detected by nano-graphene oxide. This is expected to be anon- or minimally invasive approach to diagnose the early-stage prostate cancer (26).

D)INFECTIOUS DISEASES:-

Viral infections:

The diagnostic tests for viral infection mainly depend on the salivary biomarkers, such as viral DNA and RNA, antigens and antibodies. At the proteomic level, there are saliva-based antibody tests to detect a virus that includes the hepatitis A virus, hepatitis B virus, hepatitis C virus, HIV-1,rubella virus and vesicular stomatitis virus mumps virus, among others (27).Moreover, dengue virus (DENV) RNA and non-structural protein 1 antigens are detectable from saliva, which provide a more effective way to diagnose dengue virus (28).

Other diseases:

So in the saliva of patients with gastric ulcer and chronic gastritis, *Helicobacter pylori* DNA are often detected to identify to spot H. Pylori Infection (29).Thus, saliva is used as an effective biochemical parameter for the diagnosis of chronic liver diseases (CLDs) and assessment of residual liver function in CLD (30).Based on recent studies, it has been proposed that salivary trehalose, which could be detected by some cell-based extended gate ion-sensitive field-effect transistor biosensors, that might provides a sensitive and direct way to screen for Alzheimer's disease (31).

Lung disease:

Pigeon breeder's disease (PBD) is interstitial lung diseases in which the antigens are derived from the pigeons (32).ELISA test done in saliva helps us to detect of *pneumococcal C polysaccharide* which is the diagnostic method for *pneumococcal pneumonia* (33).Lung cancers also have a higher incidence rate. Mutations identified in the EGF receptor (EGFR) are tumour-specific biomarkers for non-small cell lung carcinoma (NSCLC). A novel core technology referred to as electric field-induced releases and will detect EGFR mutations in bodily fluids that's shown to be effective, accurate, rapid and price effective for the detection of EGFR mutations within the saliva of patients with NSCLC (34).

E)HORMONAL DISEASE:-

Diabetes mellitus:

Diabetes is a metabolic disease i.e., caused by insufficient insulin secretion, insulin action or insulin resistance, that leads to glucose metabolism disorder. A positive correlation was found between α-2-macroglobulin and HbA1c, which demonstrated that the levels of α-2-macroglobulin in the saliva could reflect the action glycaemia control in patients with type 2 diabetes mellitus (35). However, the concentration of salivary melatonin decreased in patients of type 2 diabetes and patients with periodontitis. This indicates that the salivary melatonin features important role in the pathogenesis of diabetes and periodontal diseases and becomes a biomarker within the diagnosis and treatment of those two diseases (36). There is a significant correlation between both HbA1cand salivary glucose concentrations and also patients with diabetes. Thus, indicates that the blood glucose concentration could be monitored by the saliva in patients with diabetes mellitus (37).

Cardiovascular disease:

Cardiovascular disease (CVD) is related to the circulatory system of the body and also includes atherosclerosis, myocardial infarction and coronary heart disease, also found that levels of salivary inflammatory cytokines including IL-1β, IL-6, TNF-α and prostaglandin E2increased significantly in both atherosclerosis and periodontal diseases (38).Acute myocardial infarction is predicted by a combination of electrocardiogram and CRP levels with a level of 80.0% sensitivity and 100% specificity. So, these data can be demonstrated on the potential use of salivary biomarkers with electrocardiogram (ECG) for the diagnosis of the acute myocardial infarct. Moreover, the level of α-2-HS-glycoprotein in saliva decreases in patients having CVD, which also indicates that, the peptidome that might provide a potential way for the early diagnosis of patients with CVD (39). Also Refer (table 2).

Saliva biomolecules is potentially rich in diagnostic indicators for both oral and systemic disorders. The seminal field of salivary diagnostics indeed has better translational and clinical potentials. Continuing advancements in high-throughput technologies have propelled this genre by revealing unprecedented insights toward understanding the salivary milieu as a reflection of the body's overall health. Correct utilization of this results may be useful not only for identifying local and systemic disorders but it guide in the modification of various therapies. Refer table 2 for further more diseases.

CONCLUSION:

Many researchers in their studies found diverse difference in the use of salivary biomolecules. Saliva contains a large collection of diverse proteins, each with distinct biological functions. Some aid in digestion and lubricating oral cavity, and others help to maintain homeostasis and act as protection against pathogenic bacteria, buffering capacity, tissue repair etc. From this present study we conclude that, Saliva as a diagnostic tool may fulfill the objectives by providing a safe and effective means to evaluate patients and personalize their treatment modalities. We hope that the result of present study may serve as a guide for further studies.

REFERENCE:-

1. HindawiDisease Markers Volume 2019, Article ID 8761860, 11 pages https:// doi.org/10.1155/2019/8761860
2. Y. H. Lee and D. T. Wong, "Saliva: an emerging biofluid forearly detection of diseases," American Journal of Dentistry,vol. 22, no. 4, pp. 241–248, 2009.
3. N. Malathi, S.Mythili, and H. R. Vasanthi, "Salivary diagnostics: a brief review," ISRN Dentistry, vol. 2014, Article ID158786, 8 pages, 2014.
4. Poornima G, Mahesh Kumar TS. Genomic alphabets of saliva as biomarker in oral cancer. J Indian Acad Oral Med Radiol2017;29:300-5
5. V. de Almeida Pdel, A. M. Grégio, M. A. Machado, A. A. DeLima, and L. R. Azevedo, "Saliva composition and functions:a comprehensive review," The Journal of Contemporary DentalPractice, vol. 9, no. 3, pp. 72–80, 2008.
6. International Journal of Oral Science (2016) 8, 133–137
7. M. Carranza, M. E. Ferraris, and M. Galizzi, "Structural andmorphometrical study in glandular parenchyma from alcoholicsialosis," Journal of Oral Pathology and Medicine,vol. 34, no. 6, pp. 374–379, 2005.
8. R. M. Nagler, O. Hershkovich, S. Lischinsky, E. Diamond, andA. Z. Reznick, "Saliva analysis in the clinical setting: revisitingan underused diagnostic tool," Journal of Investigative Medicine,vol. 50, no. 3, pp. 214–225, 2002.
9. Mittal S et al., The diagnostic role of Saliva - A Review. J Clin Exp Dent 2011; 3(4):e314-20.
10. Sindhu S, Jagannathan N. Saliva: A cutting edge in diagnostic procedures.J Oral Dis 2014;2014:8.
11. Devi TJ. Saliva – A potential diagnostic tool. IOSR J Dent Med Sci 2014; 13:52-7. Available from: http://www.iosrjournals.org. [LastAccessed on 2018 Sep 19]
12. Journal of Oral and Maxillofacial Pathology | Volume 22 | Issue 3 | September - December 2018
13. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, *et al*. (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA.*Science* 245:1066-1073.
14. Davis PB (1987). Pathophysiology of cystic fibrosis with emphasison salivary gland involvement. *J Dent Res* 66:667-671.
15. Aubert B, Cochet C, Souvignet C, Chambaz EM (1990). Saliva from cystic fibrosis patients contains an unusual form of epidermal growth factor. *Biochem Biophys Res Commun* 16:1144-1150.
16. al-Bayaty HF, Aldred MJ, Walker DM, Newcombe RG, Swift G,Smith PM, *et al*. (1989). Salivary and serum antibodies togliadin in the diagnosis of celiac disease. *J Oral Pathol Med* 18:578-581.
17. Rujner J, Socha J, Barra E, Gregorek H, Madalinski K, Wozniewicz B, *et al*. (1996). Serum and salivary antigliadin antibodies and serum IgA anti-endomysium antibodies as a screening test for coeliac disease. *Acta Paediatr* 85:814-817.
18. Carlson AD, Obeid JS, Kanellopoulou N, Wilson RC, New MI (1999). Congenital adrenal hyperplasia: update on prenatal diagnosis and treatment. *J Steroid Biochem Mol Biol* 69:19-29.
19. Schiødt M, Thorn J (1989). Criteria for the salivary component of Sjögren's syndrome. A review. *Clin Exp Rheumatol* 7:119-122
20. Talal N (1992). Sjögren's syndrome: historical overview and clinical spectrum of disease. *Rheum Dis Clin North Am* 18:507-515.
21. Arif S, Qudsia S, Urooj S et al. Blueprint of quartz crystal microbalance biosensor for early detection of breast cancer through salivary autoantibodies against ATP6AP1. Biosens Bioelectron 2015; 65: 62–70.
22. Brooks MN, Wang J, Li Y et al. Salivary protein factors are elevated in breast cancer patients. Mol Med Rep 2008; 1(3): 375–378.
23. Agha-Hosseini F, Mirzaii-Dizgah I, Rahimi A. Correlation of serum and salivary CA15-3 levels in patients with breast cancer. Med Oral Patol Oral Cir Bucal 2009; 14 (10): e521–e524.
24. Zerah M, Pang SY, New MI (1987). Morning salivary 17-hydroxyprogesterone is a useful screening test for nonclassical 21- hydroxylase deficiency. *J Clin Endocrinol Metab* 65:227-232.
25. Chien DX, Schwartz PE (1990). Saliva and serum CA 125 assays for detecting malignant ovarian tumors. *Obstet Gynecol* 75:701- 704.
26. Hizir MS, Balcioglu M, Rana M et al. Simultaneous detection of circulating oncomiRs from body fluids for prostate cancer staging using nanographene oxide. ACS Appl Mater Interfaces 2014; 6(17): 14772–14778.
27. Parisi MR, Soldini L, Vidoni G et al. Point-of-care testing for HCV infection: recent advances and implications for alternative screening. New Microbiol 2014; 37(4): 449–457.
28. Korhonen EM, Huhtamo E, Virtala AM et al. Approach to non-invasive sampling in dengue diagnostics: exploring virus and NS1 antigen detection in saliva and urine of travelers with dengue. J Clin Virol 2014; 61(3): 353–358.
29. Yang K, Li Y, Zhou X. [Overview of researches for Helicobacter pylori in oral cavity and stomach]. Huaxi Kouqiang Yixue Zazhi 2014; 32(3): 314–318.
30. Tripathi A, Tiwari B, Patil R et al. The role of salivary caffeine clearance in the diagnosis of chronic liver disease. J Oral Biol Craniofac Res 2015; 5(1): 28–33.
31. Lau HC, Lee IK, Ko PW et al. Non-invasive screening for Alzheimer's disease by sensing salivary sugar using Drosophila cells expressing gustatory receptor (Gr5a) immobilized on an extended gate ion-sensitive field-effect transistor (EG-ISFET) biosensor. PLoS One 2015; 10(2): e0117810.
32. Mendoza F, Baltazares M, Ramirez A, Sansores R, Nava A, Banales JL, *et al*. (1996). Detection of salivary and seric IgG and IgA antipooled pigeon sera activities in patients with pigeon breeder's disease. *J Clin Lab Anal* 10:149-154.
33. Krook A, Fredlund H, Holmberg H (1986). Diagnosis of pneumococcal pneumonia by detection of antigen in saliva. *Eur J Clin Microbiol* 5:639-642.
34. Wei F, Lin CC, Joon A et al. Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer. Am J Respir Crit Care Med 2014; 190(10): 1117–1126.
35. Aitken JP, Ortiz C, Morales-Bozo I et al. α-2-macroglobulin in saliva is associated with glycemic control in patients with type 2 diabetes mellitus. Dis Markers 2015;2015: 128653
36. Abdolsamadi H, Goodarzi MT, Motemayel FA et al. Reduction of melatonin level in patients with type II diabetes and periodontal diseases. J Dent Res Dent Clin Dent Prospects 2014; 8(3): 160–165.
37. Satish BN, Srikala P, Maharudrappa B et al. Saliva: a tool in assessing glucose levels in diabetes mellitus. J Int Oral Health 2014; 6(2): 114–117.
38. Kosaka T, Kokubo Y, Ono T et al. Salivary inflammatory cytokines may be novel markers of carotid atherosclerosis in a Japanese general population: the Suita study. Atherosclerosis 2014; 237(1): 123–128.
39. Zheng H, Li R, Zhang J et al. Salivary biomarkers indicate obstructive sleep apnea patients with cardiovascular diseases. Sci Rep 2014; 4: 7046.