Literature Review

MedInform

DOI: 10.18044/MEDINFORM.2024111.1793

ISSUE 1, 2024

Saliva as potential diagnostic tool – composition, advantages, diagnostic methods and significance in diseases

Angel Adamov, Konstantin Stamatov

Department of Dental, Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Medical university-Sofia

Abstract

The advantages of saliva as a potential diagnostic fluid and the abundant salivary composition provide opportunities for easy and accessible examination of salivary content changes in a number of oral and general diseases. The analytical methods of mass spectrometry, matrix assisted laser desorption/ionization, enzyme linked immunoassay and immunofluorescence enable the identification of a large number of biomolecules. Alterations in the expression of signaling and immunoreactive molecules in cases of oral potentially malignant disorders and oral squamous cell carcinoma, where some cytokines show potential to be useful as diagnostic and prognostic factors, are of particular interest.

Keywords: saliva, cytokines, diagnostic significance

Introduction

The saliva is unique biofluid at the border between the external environment and internal medium of the body. It is important for maintaining the health and integrity of the oral cavity [10].

The rich salivary composition provides a solid basis for studies aiming composition and concentration changes of particular biomolecules. There has been wide interest in the last 10-15 years in this direction of non-invasive methods such as salivary proteins research [11,21].

Many specific biomolecules in saliva - RNA, signaling proteins, receptor molecules, antibodies, cytokines and hormones are subjects of active clinical-molecular studies [24] as potential biomarkers.
Biomarkers are molecules indicating for both normal and pathological processes and could be useful in detection, diagnosis and prognosis of given disease [21].

Saliva origin, composition and diagnostic properties

Origin

Saliva is formed by three pairs of major salivary glands (parotid, submandibular and sublingual) and numerous small salivary glands in the oral cavity mucous membrane with secretion and resorption stages. The physiological amount ranges 0.25-0.35 ml/min for unstimulated and 1-3 ml/min for stimulated secretion. Amounts below 0.1 ml/min at rest and 0.7 ml/min under stimulation are considered hyposalivation. [10]
Saliva secretion has individual variations, depending on age, gender, dietary habits, some diseases and medications [10,18].
In the glandular acini saliva is formed isotonic to the blood plasma and become hypotonic passing through the glandular ductal system [18].

Composition

Saliva contents predominantly water - up to 99%. About 1% of the composition are electrolytes and organic substances – urea, urates, bilirubin, creatinine, lipids, amino acids, sugars, cytokines and signaling molecules, enzymes, hormones and other proteins. Some drugs can be emitted with the saliva [18]. Stimulated saliva has higher levels of Na^+ and Cl^- and lower levels of K^+ and PO_4^{3-} [12]. The plasma-derived proteins amount [32] is different in stimulated and unstimulated secretion. Regarding hormones daily fluctuations in saliva levels are observed [8].
The protein amount in saliva is 1.5-2 mg/ml [39] and includes enzymes, salivary globulins, staterin, haptoglobin, cytokeratins, cystatin C, calgranulin, transferrin, annexin, glycoproteins, mucins [13,**Error! Reference source not found.**].
The antibodies in saliva are mainly of IgA class. IgM and IgG antibodies have also been found, but are considered to originate from the crevicular fluid [10].
Enzymes with protective functions are lysozyme, lactoferrin and lactoperoxidase. Histatins, possessing antibacterial and antifungal activity, also play a protective role [11,22].
The digestive enzyme α-amylase, occupying 40-50% of the total protein, initiates the breakdown of high-molecular polysaccharides [10].
Salivary composition is significantly enriched in the oral cavity. The composition of the so-called “whole mouth saliva” (WMS) is a mixture of the salivary glands secretion with the crevicular fluid and diverse complex of bioproducts of the oral microflora, metabolites, transudates, free cellular elements and microorganisms [22,10,21,11,18].
Serum proteins may be of normal salivary composition coming by active cellular transport or by passive diffusion. Nuclear acids and proteins of local origin are released during cell death or secreted by cells of the covering epithelium, tumor cells or immune cells [16,25].

Diagnostic properties

Saliva contains abundance of hormones, antibodies, growth factors and antimicrobial agents. Many of them enter the saliva from the blood plasma, passing paracellularly or transcellularly through the acinar cells of the salivary glands (fig.1).

At the same time saliva is in direct contact with pathological processes in the oral cavity. These circumstances give reason for some authors to define saliva as kind of "liquid biopsy" [18,21].

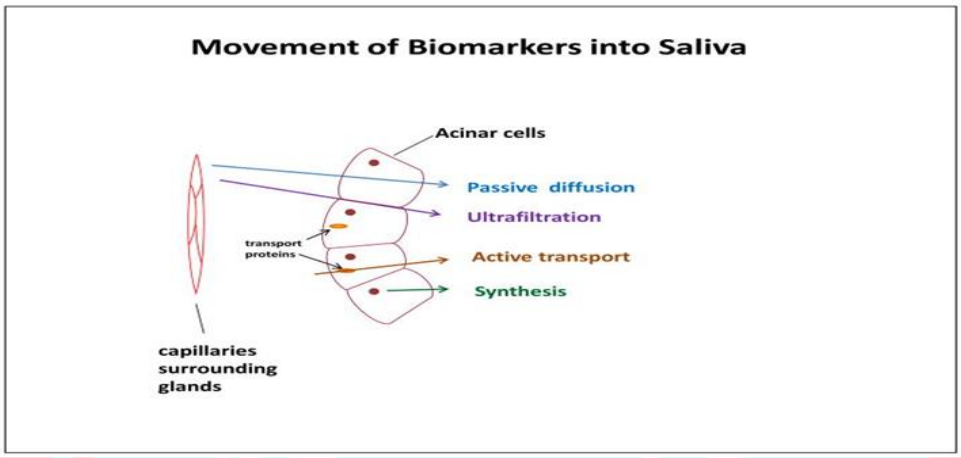

Fig.1 Movement of biomarkers into saliva (from Granger et al. [16])

Laboratory tests of blood derivatives, urine and tissue biopsies generally have standardized protocols for sample collection, storage, transportation and testing [3].

The following advantages are highlighted in saliva tests [37]:
1) easily obtained substrate;
2) non-invasive sampling;
3) no risk of introducing infection;
4) no need for tissues puncture;
5) minimal discomfort for the patient;
6) easy multiple sampling;
7) qualified staff is not required;
8) minimal requirements for samples collection, storage and transportation;
9) less demanding laboratory processing and preparation;
10) minimized economic costs;

Saliva testing methods

Laboratory processing techniques

During the laboratory tests of the saliva samples, there are two fundamentally different approaches [7,21].
1) top-down – the samples are examined without or with minimal preliminary preparation;
2) bottom-up – the sample is subjected to preliminary enzymatic degradation of proteins into peptides; this approach is referred to by the term "shotgun proteomics";

Laboratory methods

After primary sample preparation, analytical methods may include liquid chromatography and gel electrophoresis to fractionate intact proteins and peptides from the sample. For further qualitative analysis mass spectrometry (MS) and tandem-mass spectrometry (MS/MS) might be used [3].

MALDI (matrix-assisted laser desorption/ionization) and ESI (electrospray ionization) ionization methods are widely used in proteomic analysis. To enhance the capacity, the Q (quadrupole, MALDI-Q) and IT (ion trap, MALDI-IT) variants are applied. To identify the separated fractions MS/MS is used (Fig. 2). Peak m/z results are compared to databases, for example MASCOT and SEQUEST [3,11].
Other analytical methods in proteomic analysis are ELISA, Western blotting and immunofluorescence methods [11,3,21].

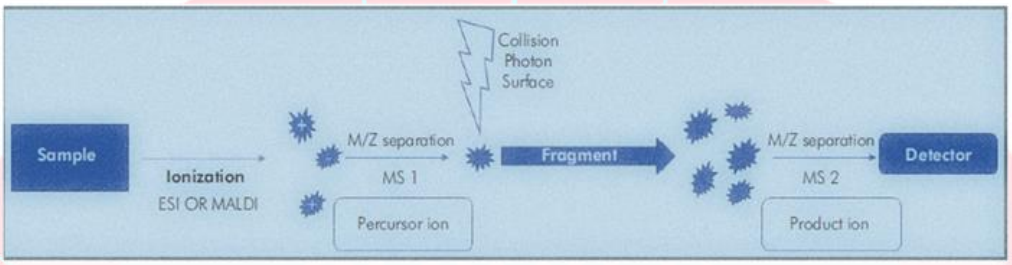

Fig.2 Tandem-mass spectrometry (from Esteves CV et al., 2019 [11])

Diagnostic properties of the salivary proteome

Huge number of research in recent years identified salivary proteins as potential biomarkers for pathological conditions, including malignant lesions [11,34].

Oral lichen planus

Increased salivary levels of the pro-inflammatory marker TNF-α have been found in erosive oral lichen planus [38]. Other studies reported increased levels of the fibrin D-fragment and the complement C3c in saliva with a concurrent decrease in cystatin SA in patients with oral lichen [33].

Caries

The salivary composition was studied in order to assess the caries risk. Differences with cases of active caries have been found in salivary protein composition, amylase levels, staterin and cystatin concentrations [34,17].

Periodontitis

Elevated levels of TNFα in saliva have been reported in cases of periodontitis [29,35]. IL6 and IL8 have been found to be elevated in periodontitis [20]. Salivary IgA, MMP-enzymes, nitrites and other biomolecules have been reported elevated in periodontitis patients [34].
Saliva composition also shows changes in other periodontal pathological conditions such as gingivitis and peri-implantitis [4,1].

Oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSSC)

Increased salivary levels of TNFα, IL1, IL6, IL8 have been found in OSCC cases [34]. It has been reported that salivary IL6 levels could differentiate OPMD from OSCC and are associated with invasive tumor growth [26]. IL8 levels are elevated in OSSC versus OPMD [28] and correlate with the tumor size [2].
TNFα salivary level correlate with the severity of the pain syndrome in OSCC patients [31]. Other studies have reported an association of TNFα levels with the degree of differentiation in OSSC and with the degree of dysplasia in OPMD [14,23].

Changes in transferrin [3] expression, some mRNA species and metabolites [34] have also been reported for OSSC.

Saliva diagnostic properties in general diseases

Tests for viral diseases, such as herpes viruses, hepatitis C viruses and human immunodeficiency virus (HIV), detect viral antigens or specific antibodies in saliva [15].

Increased levels of salivary amylase, potassium and total protein have been found in saliva samples from patients with diabetes mellitus [27].

In cases with acute myocardial infarction an increase in troponin, creatine kinase and C-reactive protein was found [5].

Rheumatoid arthritis is associated with an increase in salivary antibodies against citrullinated peptides [6].

An increase in salivary inflammatory cytokines and IgA has been found in cases of psoriasis [19].

An increase in IL-8 has been found in tumor diseases and intestinal inflammatory conditions, MMP8 has increased levels in patients with diabetes and those after cardiac operations [30].

Conclusion

Saliva is a biofluid with rich protein and metabolic composition. Saliva testing has the advantages of being a non-invasive, undemanding and relatively inexpensive method. Examination of the salivary proteome is a contemporary direction searching for diagnostic and prognostic markers in oral and general diseases. Variations in saliva content in oncological oral diseases are of particular interest. Some cytokines have been found to be associated with tumor characteristics and to show potential role, but their validation as markers needs more evidences. Despite the large number of studies, currently biomolecules that can be accepted as biomarkers have not been identified yet.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

References

1. Alsaykhan K, Khan NS, Aljumah MI, Albughaylil AS. *Comparative Evaluation of Salivary Enzyme in Patients With Gingivitis and Periodontitis: A Clinical-Biochemical Study*. Cureus. 2022 Jan 6;14(1):e20991. doi: 10.7759/cureus.20991. PMID: 35004095; PMCID: PMC8735708.
2. Azizi A, Dabirmoghadam P , Keykha F. *Comparison of the Concentration of Salivary IL8 in Ppatients with Oral Squamous Cell Carcinoma and Healthy Subjects*. J Res Dentomaxillofac Sci.2016;1(3):28-32.
3. Barbosa EB et al. *Proteomics: methodologies and applications to the study of human diseases.* Rev Assoc Med Bras (1992). 2012 May-Jun;58(3):366-75. English, Portuguese. PMID: 22735231.
4. Bolyarova, T., Petkova S. *Determination of concentration levels of some markers of inflamation and destruction in saliva and gingival exudate for diagnosing periodontal diseases*. Gen medicine, 2016; XVIII(4): 56-65.
5. Bolyarova-Konova T., M. Boncheva, M. Sandova, et al. *Comparative Investigation of Biomarkers for Acute Myocardial Infarction in Biological Fluids Serum and Saliva*. Gen Medicine, 2020; 22(1): 25-30.
6. Bolyarova-Konova, T., Stefanov, L., Kolarov, Z., et. al. (2020). *ACPA in saliva and their association with periodontitis and rheumatoid arthritis.* Rheumatology (Bulgaria), 28 (1), 20-30. https://doi.org/10.35465/28.1.2020.pp20-30
7. Cabras T, Iavarone F, Manconi B, et al. *Top-down analytical platforms for the characterization of the human salivary proteome.* Bioanalysis. 2014 Feb;6(4):563-81. doi: 10.4155/bio.13.349. PMID: 24568357.

8. Chiappin S, Antonelli G, Gatti R, De Palo EF. *Saliva specimen: a new laboratory tool for diagnostic and basic investigation*. Clin Chim Acta. 2007 Aug;383(1-2):30-40. doi: 10.1016/j.cca.2007.04.011. Epub 2007 Apr 25. PMID: 17512510.
9. Cuevas-Córdoba B, Santiago-García J. Saliva: a fluid of study for OMICS. OMICS. 2014 Feb;18(2):87-97. doi: 10.1089/omi.2013.0064. Epub 2014 Jan 3. PMID: 24404837.
10. de Almeida Pdel V, Grégio AM, Machado MA, et al. *Saliva composition and functions: a comprehensive review*. J Contemp Dent Pract. 2008 Mar 1;9(3):72-80. PMID: 18335122.
11. Esteves CV, Campos WG, Souza MM, et al. *Diagnostic potential of saliva proteome analysis: a review and guide to clinical practice*. Braz Oral Res. 2019;33:e043. doi: 10.1590/1807-3107bor-2019.vol33.0043. Epub 2019 May 16. PMID: 31508727.
12. Farnaud SJ, Kosti O, Getting SJ, Renshaw D. *Saliva: physiology and diagnostic potential in health and disease*. ScientificWorldJournal. 2010 Mar 16;10:434-56. doi: 10.1100/tsw.2010.38. PMID: 20305986; PMCID: PMC5763701.
13. Frenkel ES, Ribbeck K. *Salivary mucins in host defense and disease prevention*. J Oral Microbiol. 2015 Dec 22;7:29759. doi: 10.3402/jom.v7.29759. PMID: 26701274; PMCID: PMC4689954.
14. G D, Nandan SRK, Kulkarni PG. *Salivary Tumour Necrosis Factor-α as a Biomarker in Oral Leukoplakia and Oral Squamous Cell Carcinoma*. Asian Pac J Cancer Prev. 2019 Jul 1;20(7):2087-2093. doi: 10.31557/APJCP.2019.20.7.2087. PMID: 31350970; PMCID: PMC6745219
15. Garg N, Boyle D, Randall A, Teng A, et al. *Rapid immunodiagnostics of multiple viral infections in an acoustic microstreaming device with serum and saliva samples*. Lab Chip. 2019 Apr 23;19(9):1524-1533. doi: 10.1039/c8lc01303a. PMID: 30806409; PMCID: PMC6478527.
16. Granger DA, et al. *Incorporating salivary biomarkers into nursing research: an overview and review of best practices*. Biol Res Nurs. 2012 Oct;14(4):347-56. doi: 10.1177/1099800412443892. Epub 2012 May 15. PMID: 22593229; PMCID: PMC3971903.
17. Guo L, Shi W. *Salivary biomarkers for caries risk assessment*. J Calif Dent Assoc. 2013 Feb;41(2):107-9, 112-8. PMID: 23505756; PMCID: PMC3825179.
18. Humphrey SP, Williamson RT. *A review of saliva: normal composition, flow, and function.* J Prosthet Dent. 2001 Feb;85(2):162-9. doi: 10.1067/mpr.2001.113778. PMID: 11208206.
19. Jiménez, C.; Bordagaray, M.J.; Villarroel, et al. *Biomarkers in Oral Fluids as Diagnostic Tool for Psoriasis*. Life 2022, 12, 501. https://doi.org/10.3390/life12040501
20. Kaczyński T, Wroński J, Głuszko P, et al. *Salivary interleukin 6, interleukin 8, interleukin 17A, and tumour necrosis factor α levels in patients with periodontitis and rheumatoid arthritis*. Cent Eur J Immunol. 2019;44(3):269-276. doi: 10.5114/ceji.2019.89601. Epub 2019 Sep 30. PMID: 31933536; PMCID: PMC6953371.
21. Khurshid Z, Zafar MS, Khan RS, et al. Role of Salivary Biomarkers in Oral Cancer Detection. Adv Clin Chem. 2018;86:23-70. doi: 10.1016/bs.acc.2018.05.002. Epub 2018 Jul 23. PMID: 30144841.
22. Khurshid Z, Zohaib S, Najeeb S, et al. Human Saliva Collection Devices for Proteomics: An Update. Int J Mol Sci. 2016 Jun 6;17(6):846. doi: 10.3390/ijms17060846. PMID: 27275816; PMCID: PMC4926380.
23. Krishnan R, Thayalan DK, Padmanaban R, et al. *Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating premalignant and malignant oral disease*. Asian Pac J Cancer Prev. 2014;15(17):7141-8. doi: 10.7314/apjcp.2014.15.17.7141. PMID: 25227804.
24. Malathi M, Rajesh E, Babu N. A, Jimson S. *Saliva as A Diagnostic Tool*. Biomed Pharmacol J 2016;9(2). Available from: http://biomedpharmajournal.org/?p=8087. dx.doi.org/10.13005/bpj/1020
25. Markopoulos AK, Michailidou EZ, Tzimagiorgis G. *Salivary markers for oral cancer detection*. Open Dent J. 2010;4:172-8. doi: 10.2174/1874210601004010172. Epub 2010 Aug 27. PMID: 21673842; PMCID: PMC3111739.
26. Niklander SE. *Inflammatory Mediators in Oral Cancer: Pathogenic Mechanisms and Diagnostic Potential*. Front Oral Health. 2021 Feb 22;2:642238. doi: 10.3389/froh.2021.642238. PMID: 35047997; PMCID: PMC8757707.

27. Pérez-Ros P, Navarro-Flores E, et al. *Changes in Salivary Amylase and Glucose in Diabetes: A Scoping Review*. Diagnostics (Basel). 2021 Mar 6;11(3):453. doi: 10.3390/diagnostics11030453. PMID: 33800850; PMCID: PMC8001770.
28. Punyani SR, Sathawane RS. *Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma*. Clin Oral Investig. 2013 Mar;17(2):517-24. doi: 10.1007/s00784-012-0723-3. Epub 2012 Apr 18. PMID: 22526890.
29. Rathinasamy K, Ulaganathan A, Ramamurthy S, et al. *Estimation of TNF-α Levels in Saliva and Serum of Patients with Periodontal Health and Chronic Periodontitis: A Case-control Study*. J Contemp Dent Pract. 2020 Feb 1;21(2):148-151. PMID: 32381818.
30. Rathnayake N, Akerman S, et al. *Salivary biomarkers for detection of systemic diseases*. PLoS One. 2013 Apr 24;8(4):e61356. doi: 10.1371/journal.pone.0061356. PMID: 23637817; PMCID: PMC3634781.
31. Salvo E, Tu NH, Scheff NN, et al. *TNFα promotes oral cancer growth, pain, and Schwann cell activation*. Sci Rep. 2021 Jan 19;11(1):1840. doi: 10.1038/s41598-021-81500-4. PMID: 33469141; PMCID: PMC7815837.
32. Slavkin HC, Fox CH, Meyer DM. *Salivary diagnostics and its impact in dentistry, research, education, and the professional community*. Adv Dent Res. 2011 Oct;23(4):381-6. doi: 10.1177/0022034511420435. PMID: 21917749.
33. Talungchit S, Buajeeb W, Lerdtripop C,et al. *Putative salivary protein biomarkers for the diagnosis of oral lichen planus: a case-control study*. BMC Oral Health. 2018 Mar 13;18(1):42. doi: 10.1186/s12903-018-0504-8. PMID: 29534707; PMCID: PMC5851270.
34. Tasoulas J, Patsouris E, Giaginis C, Theocharis S. *Salivaomics for oral diseases biomarkers detection*. Expert Rev Mol Diagn. 2016;16(3):285-95. doi: 10.1586/14737159.2016.1133296. Epub 2016 Jan 11. PMID: 26680995.
35. Varghese SS, Thomas H, et al. *Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients*. Contemp Clin Dent. 2015 Sep;6(Suppl 1):S152-6. doi: 10.4103/0976-237X.166816. PMID: 26604566; PMCID: PMC4632215.
36. Vohra P, Belkhode V, Nimonkar S, et al. *Evaluation and diagnostic usefulness of saliva for detection of HIV antibodies: A cross-sectional study*. J Family Med Prim Care. 2020 May 31;9(5):2437-2441. doi: 10.4103/jfmpc.jfmpc_138_20. PMID: 32754516; PMCID: PMC7380795.
37. Zhang A, Sun H, Wang X. *Saliva metabolomics opens door to biomarker discovery, disease diagnosis, and treatment*. Appl Biochem Biotechnol. 2012 Nov;168(6):1718-27. doi: 10.1007/s12010-012-9891-5. Epub 2012 Sep 13. PMID: 22971835.
38. Zhu ZD, Ren XM, Zhou MM, et al. *Salivary cytokine profile in patients with oral lichen planus*. J Dent Sci. 2022 Jan;17(1):100-105. doi: 10.1016/j.jds.2021.06.013. Epub 2021 Jul 7. PMID: 35028026; PMCID: PMC8739733.
39. Zin, Thaw Htet,; Soe, Ommar; Thet, Yin Mu et al. Salivary total protein levels among healthy controls, chronic gingivitis patients and chronic periodontitis patients. Journal of Oral Research and Review 13(1):p 18-24, Jan–Jun 2021. | DOI: 10.4103/jorr.jorr_46_20

Corresponding author:

Angel Adamov;
a.adamov@fdm.mu-sofia.bg
Department of Dental, Oral and Maxillofacial surgery, Faculty of Dental Medicine, Medical University- Sofia, Sofia, Bulgaria

Adamov A, Stamatov K. Saliva as potential diagnostic tool – composition, advantages, diagnostic methods and significance in diseases. Medinform 2024; 11(1):1793-1799.