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 PO₄³⁻ [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])](image)

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])](image)

**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 OSSC cases [34]. It has been reported that salivary IL6 levels could differentiate OPMD from OSSC 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 OSSC 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**


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