



Saliva: Newer Avenues in the Era of Molecular Biology, Diagnostic and Prognostic Application

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The salivary fluid has an old history of study, but its physiological importance has only been recognized recently. In the past 50 years, the pace of salivary research has accelerated with the advent of new techniques that illuminated the biochemical and physicochemical properties of saliva. The interest in saliva increased, further, with the finding that saliva is filled with hundreds of components that might serve to detect systemic diseases and/or act as an evidence of exposure to various harmful substances as well as provide biomarkers of health and disease. The role of saliva in the diagnosis as well as monitoring of glycemic control has, also, been attracting attention of clinical researchers in recent times although results have been conflicting. To conclude, saliva is a whole, diverse fluid that serves various purposes discussed in detail in the literature. The recent introduction of molecular biology opens up, once again, new vistas and a new search of the role of salivary fluid as a potential diagnostic tool which has an added advantage of being noninvasive. The present review presents such insight into the possible use of salivary fluid as a potential diagnostic and prognostic tool for the search of numerous diseases as well as for monitoring the treatment outcomes and assesses prognosis in such varied states of derangements of metabolic functions.

Key words: Saliva, diagnostics, systemic diseases, forensic applications

INTRODUCTION

The salivary fluid is an exocrine secretion consisting of approximately 99% water with a variety of electrolytes including sodium, potassium, calcium, magnesium, chlorides, bicarbonates, phosphates, and proteins represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, and traces of albumin with glucose and nitrogenous products such as urea and ammonia secreted mainly by three pairs of major salivary glands, namely parotid, submandibular, and sublingual glands. A plethora of minor salivary glands distributed over the buccal mucosa, lips, and along the mucosa of the upper aerodigestive tract present from

the nasal cavity to the larynx and pharynx, also, participate in this secretion. Together, they are responsible for the remaining 5% of saliva secreted in humans.¹⁻⁴ It is considered that humans secrete approximately 0.5 L of saliva per day in response to stimulation of the sympathetic and parasympathetic sections of the autonomic nervous system.³⁻⁵ Whole saliva is a multiglandular secretion complex consisting of gingival fluid, desquamated epithelial cells, microorganisms and products of their metabolism, food debris, leukocytes, and mucus from the nasal cavity and the larynx and pharynx. Saliva has varied functions from tissue repair to protection, digestion, taste, and

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antimicrobial action, in the maintenance of tooth integrity and antioxidant defense system.^{6,7} The average daily volume of saliva production is 500–1000 ml with the submandibular gland producing around 70% of the total volume, parotid contributing for 25%, and the sublingual gland contributing to about 5% of the total salivary secretion. The contribution of minor salivary glands toward the total volume of saliva, although, has more or, less local effects.^{3,4} The functions of saliva with the split-up of the various individual constituents are summarized in Table 1 while the methods of collection of resting/unstimulated and stimulated saliva are summarized in Table 2.

SALIVA AS DIAGNOSTIC FLUID

The salivary fluid has an old history of study, but its physiological importance has only been recognized recently. In the past 50 years, the pace of salivary research has accelerated with the advent of newer techniques that have illuminated the biochemical and physicochemical properties of saliva. The interest in saliva increased, further, with the finding that saliva is filled with hundreds of components that might serve to detect systemic diseases and/or act as an evidence of exposure to various harmful substances and provide biomarkers of health and disease.⁹⁻¹² Many researchers have made use of sialometry and sialochemistry to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relationship between oral and systemic health. However, since several factors can influence salivary secretion and composition, a strictly standardized collection must be made, so the above-mentioned examinations are able to reflect the real functioning of the salivary glands and serve as an efficient means for monitoring the systemic illnesses and health.¹³⁻²⁵ The aim of the present literature review was to present such insight into the possible use of salivary fluid as a potential diagnostic and prognostic tool for the search of numerous diseases as well as for monitoring

the treatment outcomes and to assess prognosis in such varied states of derangements of metabolic functions apart from investigating the composition and functions of saliva as well as to describe the factors that influence salivary flow and its biochemical composition.

SERUM VERSUS SALIVA

Currently, sera samples are used for the diagnosis and for monitoring the control of the disease processes and assessing the prognosis for most of the diseases. However, collection of sera samples has its own disadvantages including being an invasive procedure, being painful, and being associated with the risk of transmission of numerous infectious disease processes in cases where a strict asepsis is not followed. Thus, a simpler screening criterion which is noninvasive is an absolute necessity to make case finding easier for the clinicians and for the frequent monitoring of the disease processes. Furthermore, the ability to monitor health status, disease onset, progression, and treatment outcomes through noninvasive means is a highly desirable goal in health-care management.⁹ Like serum, saliva is a complex biological adjunct containing a variety of hormones, antibodies, enzymes, antimicrobial, and growth factors. Many of these enter saliva from the serum by passing through the spaces between the cells by transcellular (passive intracellular diffusion and/or active transport) or paracellular (extracellular ultrafiltration) routes. Therefore, most of the components found in the serum are, also, present in saliva, thus making saliva functionally equivalent to serum in reflecting the physiological status of the body, including the hormonal, nutritional, and various metabolic variations.¹³ The pace of research in relation to the salivary diagnostics and proteomics, however, could not reach the extent that was expected with the advent of newer techniques in the recent decades. The major problems in clinical salivary diagnostics are attributed mainly due to nonstandardized collection procedures and difficulty in interpretations caused due to the great diurnal variations of salivary secretion and the individual differences, in general. The major advantages of using saliva as a diagnostic fluid are its noninvasiveness, ease of collection, no requirement of special equipments and/or trained staff, and its usefulness in blood dyscrasias along with a likely better compliance with the children and geriatric patients.⁹⁻¹¹

SALIVARY ANALYSIS AS A DIAGNOSTIC TOOL IN VARIOUS PATHOLOGIC CONDITIONS

Analysis of saliva is done commonly for the diagnosis of the following conditions:¹³⁻²⁵

- Hereditary diseases
 - Cystic fibrosis^{15,16}
 - 21-hydroxylase deficiency.

Table 1: Functions of saliva and salivary components⁷

Function	Constituents
Tissue repair	EGF
Protection	Glycoproteins and water
Tamponage	Phosphates, bicarbonates, and proteins
Digestion	Mucin, bicarbonates, amylase, proteases, and lipases
Gustation	Water, gustine, and Zn ⁺⁺
Antimicrobial action	Glycoproteins, IgA, IgM, IgG, lysozyme, lactoferrin, peroxidase, cystatin, mucin, and histatin
Tooth integrity	Calcium, phosphates, proteins rich in proline, and staterine
Antioxidant defense system	Uric acid, albumin, alpha-tocopherol, beta-carotene, glutathione, and ascorbic acid

EGF=Epidermal growth factor; IgA=Immunoglobulin A; IgM=Immunoglobulin M; IgG=Immunoglobulin G

Table 2: Methods of collection of saliva^{6,8}

Collection of resting/unstimulated whole saliva ^{6,8}			
Methods	Measurement technique	Advantages	Disadvantages
Passive drainage	Saliva flows passively from the mouth into a graduated container	Reproducible Reliable to measure salivary flow	Evaporation of saliva
Active drainage	The saliva accumulated in the mouth is regularly expectorated into a graduated container	Reproducible Reliable to measure salivary flow	Evaporation of saliva There is a small stimulation of salivary flow in the act of spitting
Suction	Saliva pooled in the floor of the mouth is vacuumed and accumulated in a graduated container	Does not depend on patient collaboration	There is a small stimulation of salivary flow
Absorption	Swab, cotton, or gauze roll, preweighed, is inserted into the mouth for a certain amount of time and later, weighed again	Detects the presence of saliva in the oral cavity, using a simple and easy method, especially in outpatient clinics and dental offices	There is a small stimulation of salivary flow Less reliable method Alters concentration of some salivary components
Collection of stimulated whole saliva ^{6,8}			
Methods	Measurement technique	Advantages	Disadvantages
Mechanical	To chew paraffin, silicone, or unflavored chewing gum and spit the saliva into a graduated container	Absence of side effects There is no contamination of saliva with exogenous substances	The frequency of mastication must be standardized with the aid of metronome
Gustatory	Applying citric acid to the lateral borders of the tongue		It can interfere with the analysis of some salivary components
Absorption	Applying citric acid to the lateral borders of the tongue	Detects the presence of saliva in the oral cavity using a simple and easy method, especially in outpatient clinics and dental offices	There is a small stimulation of salivary flow Less reliable method Alters the concentration of some salivary components

- Infectious diseases: Saliva contains immunoglobulins (IgA, IgM, and IgG) that originate from two sources: the salivary glands and serum. Antibodies against viruses, bacteria, fungi, and parasite can be detected in saliva and can aid in the diagnosis of the following infections:
 - *Helicobacter pylori*^{17,18}
 - *Shigella*
 - Pneumococcal pneumonia
 - Lyme disease
 - *Taenia solium*
 - *Entamoeba histolytica*
 - *Mycobacterium tuberculosis*¹⁹
 - Human immunodeficiency virus^{20,21}
 - Hepatitis
 - Measles²²
 - Mumps
 - Rubella
 - Rotavirus
 - Herpes simplex virus-1
 - Dengue
 - Monitoring of levels of hormones^{23,24}
 - Cortisol
 - Aldosterone
 - Testosterone
 - Dehydroepiandrosterone
 - Progesterone
 - Insulin
 - Hormones associated with bone turnover²⁵
 - Salivary osteocalcin and pyridinoline in osteopenia and osteoporosis for bone mineral density (BMD)
 - BMD/t-scores
 - Autoimmune diseases
 - Sjogren's syndrome
 - Malignancies²⁶⁻³⁷
 - p53 and CA 125 antibodies in oral squamous cell carcinoma (OSCC)^{27,28}
 - Salivary defensin-1 in OSCC
 - Cancer antigen 15-3 (CA15-3) and c-erbB-2 (erb) and in breast carcinoma^{33,34}
 - CA 125 in epithelial ovarian carcinoma³⁵
 - Prostate-specific antigen in prostatic adenocarcinoma³⁶
 - High levels of nitrates in carcinoma of the digestive tract.
- The oral manifestations of leukemias, also, occur early in the course of disease and these oral features can at times act as diagnostic indicators. A rise in salivary amylase levels in leukemic patients has, also, been reported.

DETECTION OF DRUGS IN SALIVA

Saliva can, also, be used as a medium to detect the presence and levels of the various drugs of therapeutic benefits and associated with drug abuse for which it plays a clinically significant role in the diagnostic and prognostic applications as is being reflected with the names of the drugs the presence of which can be and is usually detected in saliva in Table 3.³⁸⁻⁴⁰

FORENSIC EVIDENCE IN SALIVA

Saliva is deposited on the skin or object surface in enough amounts to allow typing of the deoxyribonucleic acid (DNA).

Table 3: Detection of drugs in saliva³⁸⁻⁴⁰

Antipyrine
Carbamazepine
Caffeine
Cisplatin
Cyclosporine
Diazepam
Digoxin
Ethosuximide
Irinotecan
Lithium
Methadone
Metoprolol
Oxprenolol
Ethanol
Marijuana
Phencyclidin
Paracetamol
Phenytoin
Primidone
Quinine
Procainamide
Sulfanilamide
Theophylline
Tolbutamide
Opioids
Amphetamines
Benzodiazepines
Barbiturates
Cocaine
Nicotine

Polymerase chain reaction (PCR) allows replication of thousands of copies of a specific DNA sequence *in vitro* enabling the study of small amounts of DNA.⁴¹⁻⁴⁴

DETECTION OF ORAL MANIFESTATIONS WITH RELEVANCE TO THE DIAGNOSIS OF SYSTEMIC DISEASES

Some systemic diseases affect salivary glands directly or indirectly and may influence the quantity and quality of saliva. These characteristic changes may contribute to the diagnosis and early detection of these diseases. Common examples of such diseases include¹³⁻²⁵

- Cystic fibrosis
- HIV infection
- Sjögren’s syndrome
- Rheumatoid diseases
- Myasthenia gravis
- Sarcoidosis
- Hormonal disorders
- Malnutrition
- Dehydration
- Vitamin deficiency
- Hypertension
- Cirrhosis
- Parkinsonism
- Renal disease
- Diabetes mellitus
- Various malignancies.²⁶⁻³⁷

SALIVA IN ORAL HEALTH AND DISEASE, DISEASE DIAGNOSTICS, AND MONITORING

Saliva contains a spectrum of immunologic and nonimmunologic proteins with antibacterial properties. In addition, some proteins are necessary for inhibiting the spontaneous precipitation of calcium and phosphate ions in the salivary glands and in their secretions. Both the acquired film and the biofilm have proteins derived from saliva. High numbers of *Streptococcus mutans* and *Lactobacillus* indicate a shift in oral microflora from healthy to more cariogenic environment. Secretory IgA is the largest immunologic component of saliva. It can neutralize viruses, bacteria, and enzyme toxins. It serves as an antibody for bacterial antigens and is able to aggregate bacteria inhibiting their adherence to oral tissues.⁴⁵ Other immunologic components such as IgG and IgM occur in lesser quantity and probably originate from gingival fluid.³ Among the nonimmunologic salivary protein components, there are enzymes (lysozyme, lactoferrin, and peroxidase), mucin

glycoproteins, agglutinins, histatins, proline-rich proteins, statherins, and cystatins.⁴⁵ Lysozyme can hydrolyze the cellular wall of some bacteria, and because it is strongly cationic, it can activate the bacterial “autolysins” which are able to destroy bacterial cell wall components. Gram-negative bacteria are more resistant to this enzyme due to the protective function of their external lipopolysaccharide layer. Other antibacterial mechanisms have, also, been proposed for this enzyme including inhibiting bacterial aggregation and adherence.⁴⁶⁻⁴⁸ Lactoferrin links to free iron in the saliva causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival such as the *S. mutans* group. Lactoferrin, also, provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions.⁴⁶⁻⁴⁹ Peroxidase or sialoperoxidase offers antimicrobial activity because it serves as a catalyst for the oxidation of the salivary thiocyanate ion by hydrogen peroxide into hypothiocyanate, a potent antibacterial substance. As a result of its consumption, proteins and cells are protected from the toxic and oxidant effects of hydrogen peroxide.⁵⁰ The proline-rich proteins, salivary mucins, and statherins inhibit the spontaneous precipitation of calcium phosphate salts and the growth of hydroxyapatite crystals on the tooth surface preventing the formation of salivary and dental calculus. They favor oral structure lubrication and it is probable that both are important in the formation of acquired film. Another function proposed for the proline-rich proteins is the capacity to selectively mediate bacterial adhesion to tooth surfaces.^{50,51} The cystatins are also related to acquired film formation and to hydroxyapatite crystal equilibrium. Due to its proteinase inhibiting properties, it is surmised that they act in controlling proteolytic activity.⁵⁰⁻⁵³ The histatins, a family of histidine-rich peptides, have antimicrobial activity against some strains of *S. mutans* and inhibit hemagglutination of the periopathogen *Porphyromonas gingivalis*.^{54,55} They neutralize the lipopolysaccharides of the external membranes of Gram-negative bacteria and are potent inhibitors of *Candida albicans* growth and development through the union of positively loaded histatins with the biological membranes resulting in the destruction of their architecture and altering their permeability.^{56,57} Other functions attributed to these peptides include participation in acquired film formation and inhibition of histamine release by the mast cells suggesting a role in the inflammatory process.⁴⁵ Salivary agglutinin, a highly glycosylated protein frequently associated with other salivary proteins and with secretory IgA, is one of the main salivary components responsible for bacteria agglutination.⁵⁰ In a healthy situation, there is no correlation between saliva secretion rate and dental caries. When the salivary secretion rate drops below a certain minimum, the predisposition for dental caries increases dramatically.⁴⁶⁻⁴⁸ Saliva secretion rate

and buffering capacity have proven to be sensitive parameters in caries prediction models.⁵⁸ Diagnostic kits for *S. mutans* and *Lactobacillus* counts are widely used in dental practice and can be conducted without laboratory facilities. Commercial kits are also available for determination of the salivary buffering capacity. Different saliva-based caries activity tests include:^{59,60}

- *Lactobacillus* colony count test
- Snyder test
- Reductase test
- Buffer capacity test
- Fosdick calcium dissolution test
- *S. mutans* adherence test
- *S. mutans* dip-slide test.

Similarly, during active periods of the periodontal disease, increased levels of inflammatory markers such as C-reactive protein (CRP), various interleukins, and matrix metalloproteinases (MMPs) can be demonstrated in the saliva.⁶¹⁻⁶³ The traditional methods to diagnose periodontal disease rely on measuring the periodontal pocket depth and clinical attachment loss and evaluating the radiographs for assessing the bone loss. These assessments do not predict periodontal disease in their earliest state.⁶⁴ Since periodontal disease is irreversible disease process, an early diagnosis is imperative. Researchers have been investigating ways to detect periodontal disease in their preclinical phases using genetic, microbial, and protein biomarkers.⁶⁵ Since the early 1990s, much research was generated to learn about the biomarkers of periodontal disease. Gingival crevicular fluid (GCF) became an early medium to examine for such biomarkers due to its location within the sulcus and easy accessibility. The major advantages of using GCF for diagnostic purpose include a site-specific approach for the detection of the presence or absence of specific periodontal pathogens, gingival and periodontal inflammation, the host inflammatory-immune response to certain pathogenic species, and the host tissue destruction. However, relatively expensive, technique sensitive, requirement of multiple samples of individual tooth sites, and laboratory processing make GCF less preferred when compared to saliva.^{66,67} Saliva is readily available and easier to collect than is GCF. Saliva, also, contains a plethora of biomarkers for periodontal disease activity including GCF and has emerged as the medium of choice to detect markers for periodontal disease.⁶⁸ Significant advances are in development for the screening of periodontal disease. Researchers have reported that high levels of the inflammatory biomarkers such as CRP, various interleukins, and MMPs including MMP-8 have been associated with chronic and aggressive periodontal disease.⁶¹⁻⁶³ MMP-8 has been identified as a major tissue destructive enzyme in periodontal disease.

Consequently, MMP-8 is a promising candidate for diagnosing and, possibly more importantly, assessing the progression of periodontal disease.⁶⁹ Similarly, serum and salivary aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase are considered to be the possible disease markers in periodontal disease.⁷⁰ It is clear that individual susceptibility along with a variety of local and systemic conditions can influence the initiation and progression of periodontal disease. Therefore, it is important that advances in diagnostic testing are made to help identify early periodontal risk. The use of saliva-based diagnostics appears promising for future application to diagnose periodontal disease and to predict the various possible treatment outcomes.^{68,69} Furthermore, several bacteria have been associated with periodontal disease which are susceptible to different antibiotics.⁵³⁻⁵⁵ Therefore, before antibiotic treatment, pathogens are determined by culturing or PCR techniques. In this, saliva plays a pivotal role apart from the GCF for which methylcellulose paper strips are used to collect fluid from the gingival crevices around the teeth. Urea, a buffer present in salivary fluid, is a product of amino acid and protein catabolism that causes a rapid increase in biofilm pH by releasing ammonia and carbon dioxide when hydrolyzed by bacterial ureases. Ammonia, a product of urea and another significant catabolic end product of amino acid metabolism, is potentially cytotoxic to gingival tissues. It is an important factor in the initiation of gingivitis and, further, periodontal disease because it increases the permeability of the sulcular epithelium to a plethora of toxic or antigenic substances in addition to the formation of low pH biofilms and plaque deposits detrimental to periodontal health.⁷¹ Furthermore, it has been shown that untreated periodontal disease can lead to systemic disorders such as cardiovascular disease and diabetes. Nevertheless, the recent focus on the potential role of periodontal disease as a risk factor for cardio- and cerebrovascular disease and diabetes brings new importance to this aspect of salivary analysis.⁷²⁻⁷⁴

Limitations of the model for using saliva in diagnostics

Xerostomia accounts for one of the major limitations for using saliva in diagnostics. Many classes of drugs, particularly those that have anticholinergic action (antidepressants, anxiolytics, antipsychotics, antihistaminics, and antihypertensives), may cause reduction in salivary flow and alter composition of saliva leading to xerostomia of varying grades and associated adverse effects.^{75,76} Numerous diseases, too, have an impact on salivary flow rates and composition, one among them being diabetes mellitus, apart from a plethora of other conditions including numerous autoimmune and/or inflammatory conditions such as Sjogren's syndrome and primary biliary cirrhosis, graft versus host disease,

IG-G4-related sclerosing disease, degenerative diseases such as amyloidosis, granulomatous conditions including sarcoidosis, infections including HIV/AIDS, hepatitis C and malignancies such as lymphomas, and salivary gland agenesis or, aplasia. In addition, patients with salivary gland changes after exposure to radiation in the head and neck area for treatment of malignancies, also, pose such challenges.^{77,78} Apart from the above-mentioned limitations, age-related degenerative changes seen in salivary glands, also, add to a significant fraction of geriatric population to suffer from xerostomia seen to varying grades.⁷⁹⁻⁸³

CONCLUSION

Saliva is a whole, diverse fluid that serves various purposes discussed in detail in the literature. Furthermore, there has been sufficient literature that assays the role of saliva as a potential diagnostic fluid although with conflicting reports and results from the various studies conducted to prove the reliability of saliva as a potential diagnostic fluid. Although the recent introduction of molecular biology opens up new vistas and a new search for the role of salivary fluid as a potential diagnostic fluid, further studies and an active search are, still, warranted to establish the role of saliva in the diagnosis of various conditions as well as its suitability and sound usage for prognostic and forensic purposes.

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Conflicts of interest

There are no conflicts of interest.

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