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The Versatile Nature of Saliva- Forefront in Prophylactic Biomarkers: A Brief Review

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Review article

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ABSTRACT

Prevention is better than cure for many human diseases which have global impact includes diabetes, cancer, cardiovascular, metabolic, and neurological diseases, and in late December of 2019, the COVID-19 triggered a rapid outbreak is also under consideration. For the early detection and diagnosis saliva serves as a potential biomarker to reduce disease severity and to prevent complications, since there is a lack of success in the development of better therapy. Saliva is a biochemical fluid mixture that offers an advantage over serum and other biological fluids by an economic and non-invasive collection method for mass screening of disease and its increased progression. Saliva and its component involved in maintaining mucosal integrity and aids in digestion through salivary enzymes. Saliva has many oral health benefits including buffering, lubrication, and re-mineralization. The salivary diagnostics into clinical practice is gaining reality and is equivalent to serum analysis. The investigative use of saliva is already applied in dental health and now it is used to monitor and analyses in various other systemic disorders and diseases. This article emphasizes the multifunctional role of saliva in contributing as a biomarker and its tremendous role as a sensitive diagnostic tool for oral, systemic, and infectious diseases. Its diagnostic roles are expecting to contribute to future pandemic control as well.

Keywords: Saliva; Salivary Diagnostics, Non-Invasive, Novel Corona Virus, COVID-19 (Corona-Virus disease), 2019-nCoV, SARS-CoV-2, Salivary Proteins, Xerostomia, Versatile Nature.

Introduction

The field of salivary diagnostics emerged in the year 1960s when Cystic fibrosis patients confirmed higher levels of calcium in saliva. In recent years, the field broadened on different levels, including the detection of cancers, heart diseases, HIV infections, and other various infectious diseases.[1] Saliva is a distinctive biological fluid that has a varying spectrum of polypeptides, proteins, nucleic acids, hormones, various enzymes, electrolytes, and microorganisms. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 6–7.[2] Most of reactive fluids that are collected for laboratory testing are blood, urine, cerebrospinal fluid, synovial, peritoneal, ascetic fluids act as effective biomarkers for the diagnosis of diseases. Blood and

urine samples are common compared to others because they require complicated procedures to aspirate from the body. The diagnostic prospective of saliva is reflected by the presence of multiple biomarkers which appears at a concentration much lesser than blood and still parallel in reflecting the body's health and wellbeing.[3] An outbreak of COVID-19 (coronavirus disease) is emerging and rapidly spreading worldwide.[4] Saliva stands at the entry of respiratory system and was also found COVID-19 nucleic acid positive.[5-8] Saliva is gaining importance day by day and is functionally equivalent to the serum maintaining a physiological state of the body, including hormonal, emotional, nutritional, and metabolic variations.[1] In recent times, the use of salivary diagnostics in a clinical setting is becoming a reality because of the improved efficiency of genomic and proteomic technologies.

Saliva Composition and Functions

Saliva is a biochemical fluid produced by three major salivary glands: parotid, submandibular, and sublingual with added secretions from minor salivary gland.[9] Saliva is secreted 90% from major salivary glands and 10% from minor salivary glands where pH ranging from 6 to 7.[10-11] The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow).[12] The major salivary glands include the parotid glands, the submandibular and sublingual glands. Minor glands that produce saliva are found in the lower lip, tongue, palate, cheeks, and pharynx.[13] Factors affecting salivary secretion include psychological factors such as pain, certain types of medication, and various local or systemic diseases affecting the glands themselves.[13, 14-15]

Whole saliva is a bio-mixture, which physiologically contains gingival crevicular fluid, microorganisms, desquamated oral epithelial cells, blood, gastric acid refluxes, food debris, and respiratory secretions in pathological event.[16] A whole mixture of saliva composed of total solids at resting and stimulated state. Total solids comprise of both organic and inorganic constituents. Organic constituents such as proteins, amino acids, amylase, lysozyme, IgA, IgG, glucose, citrate, lactate, ammonia, urea, uric acid, creatinine, cholesterol, cAMP and inorganic constituents such as sodium, potassium, thiocyanate, calcium, phosphate, chloride, fluoride.[17] These components interact in related function in the following general areas: (1) bicarbonates, phosphates, and urea act to modulate pH and buffering action of saliva; (2) macromolecule such as proteins and mucins serve to cleanse, aggregate, and attach oral microbes and involve in dental plaque metabolism; (3) minerals such as calcium, phosphate, and proteins work together in antisolubility factor and modulate demineralization and remineralization; and (4) proteins, immunoglobulins, and various enzymes serves antibacterial action.[18]

Sl. No.	Salivary components	Functions
1.	Amylase	Digestive functions - role in the digestion of carbohydrates
2.	Bacterial and PMN proteases	Affecting the integrity of the mucous membranes and causing ulceration
3.	Bicarbonate, phosphate, and histidine-rich peptides	Buffering action
4.	Calcium, phosphorus, magnesium, and fluoride	Maintenance of Tooth Integrity
5.	Cysteine-containing phosphoproteins	Antiprotease activity
6.	Lactoferrin	Compete with the bacterial iron-chelating molecules, capable of a bactericidal effect
7.	Lysozyme	Cause lysis of bacterial cells, especially Streptococcus mutants
8.	Mucin glycoproteins	-Lubrication and demulcent properties- when complexed with albumin, was also an extremely effective lubricant

		-Maintenance of Mucous Membrane Integrity- serves as natural "waterproofing" and helps to maintain these tissues in a hydrated state
9.	Nerve growth factor and epidermal growth factor	Accelerate wound-healing
10.	Polypeptide hormone- urogastrone.	Gastric cytoprotection and inhibition of gastric acid secretion
11.	Salivary peroxidase	Oxidation of salivary thiocyanate by hydrogen peroxide (generated by oral bacteria) to hypothiocyanite and hypothiocyanous acid, enhanced by interaction with secretory IgA.
12.	Secretory IgA	-Inhibit bacterial attachment
13.	Urea	Neutralize the acid.

Table 1: Multiple Functions of Saliva: [19-30]

Collection of Saliva for Mass Screening

Usually, saliva is collected under-stimulated and unstimulated conditions. Stimulated whole saliva is collected by masticatory action-chewing paraffin wax or by gustatory stimulation with the aid of acetic acid in the mouth followed by a collection of saliva. Unstimulated saliva is collected by drooling the saliva in the mouth for a minute and draining it in a wide bore sterile vessel or by suction methods or by swabbing method.[31]

Recently, new methods have been developed for the collection of saliva-based on the modification of the old expectoration techniques.[32] Oragene is a sophisticated technique used commonly in the preservation of buffers to protect the integrity of the sample for further processing and extractions. Saligene, oracol, and verofy are other saliva expectoration methods. Saligene is a spit in a cup technique alternative to the traditional techniques. In this technique, saliva is expectorated into a collection tube up to predetermined measurement following which a plunger is used to cap the tube. For further processing, the pressure is released by the buffer into the specimen. Oracol is based on absorbent foam swab which picks up 1mL of saliva. Verofy is a unique method of obtaining immediate results which utilizes high-quality immunochromatographic strips .[33]

Noninvasive nature, less hazardous and secure to health care workers, salivary specimen collection for diagnosis of various diseases and including pandemic coronavirus disease has more advantages and acceptable for patients. Till now, three approaches have been reported to collect saliva—coughing out, saliva swabs, and directly from salivary gland duct.[5]

Diagnostic Use of Saliva over Serum

The biochemical components present in the whole saliva holds a lot of importance in early detection and diagnosis of the diseases because of the following advantages over Serum: [1, 34-36]

1. Non-invasive collection of sample, easy to handle, cost effectiveness, inexpensive
2. Safer to administer than serum sampling (no needles)
3. Real-time diagnostic values
4. No required trained medical staff
5. Smaller sample aliquots, multiple samples can be obtained easily
6. Collection and screening can be done at home, good cooperation with patients
7. Minimal risks of cross-contamination, easy storage and transportation
8. More economical sampling, greater sensitivity
9. Requires less manipulation compared to serum during diagnostic procedures
10. Commercial availability of screening assays [37]

The molecules of saliva have extensive diagnostic values in detecting the onset and severity of various diseases/conditions. Promising new technologies have unveiled large numbers of medically valuable salivary biomarkers for clinical diagnostic applications.[38]

Biomarkers	Infections/ Diseases/ Conditions	Source of Biomarkers
ACE2 receptor, furin	Coronavirus disease	Saliva
Blood group antigens and DNA testing	Forensic evidence	Saliva
Cardiac troponins, C-reactive protein, myoglobin, myeloperoxidase, ICAM-1,CD40, and salivary lysozyme	Cardiovascular Diseases	Serum and saliva
Cathepsin-D, sodium, potassium, chloride, calcium, magnesium, and lactate dehydrogenase Inorganic constituents, total protein	Genetic disorders (1) Cystic fibrosis (2) Ectodermal dysplasia	Saliva
Cortisol, nitrite, uric acid, sodium chloride, pH, alpha-amylase, and lactoferrin. Salivary phosphate, serum creatinine, and glomerular filtration rate	Renal diseases	Serum and saliva
D-PYR, OC concentration, calcaneus T scores, hepatocyte growth factor, interleukin-1beta, salivary osteonectin, and ALP activity	Bone growth & remodeling	Serum and saliva
Lactoferrin, beta 2 microglobulin, lysozyme C, cystatin C, salivary amylase, and carbonic anhydrase IgA production Alpha-amylase and kallikrein	Autoimmune diseases (1)Sjogren's syndrome (2)Multiple sclerosis (3)Sarcoidosis	Saliva
Measles virus- specific IgM HIV—HIV-1,HIV-2—antibodies, salivary proteins Mycobacterium tuberculosis, MUC5B, and MUC7 Candidiasis immunoglobulins, Hsp70, and calprotectin, histatins, mucins, basic proline rich proteins, and peroxidases	Infections (1)Viral infections (2)Bacterial infections (3)Fungal infections	Serum and saliva
lncRNA, miRNA, CCNI, EGFR, FGF19, FRS2 and GREB1, AGPAT1, B2M, BASP2, IER3, and IL1B, p53, CA15-3, C-erb2, CA125, FGF2, PSA, cortisol, silver nitrate and nitrite, and salivary adenosine deaminase	Malignancy	Serum and saliva
Nicotine, cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepines, amphetamines, ethanol, cotinine, methamphetamine, endogenous □-hydroxybutyricacid, and 3,4-methylene dioxy methamphetamine	Drug level monitoring	Serum and saliva
Salivary amylase, cortisol, substance P, lysozyme, secretory IgG, and testosterone	Psychological conditions	Saliva
Streptococcus mutants and lactobacilli count, aspartate aminotransferase, alkaline phosphatase, uricacid, albumin, pIgR, Arp3, CAVI, IL-1Ra, PLS-2, LEI, and IGJ	Dental caries and periodontal diseases	Saliva

Table 2: Salivary Biomarkers in Various Infections/Diseases/Conditions.[39]

All these studies enhance that saliva as a potential diagnostic tool for the identification and analysis of several biomarkers in infants, children, adults and uncooperative patients.[40]

With the advent of improved efficiency and accuracy of proteomic, genomic, transcriptome analysis salivary diagnostics turn into a clinical and commercial reality.[1] Advanced molecular technique PCR led to the use of saliva as a source of microbial DNA for the detection of bacteria and virus.[41] Saliva is increasingly used in DNA analysis as it serves as a useful source of biomarker profiling of oral or systemic diseases, and forensic identification.[42,43] DNA tests are also a method of detection of HIV infection by recognizing the viral sequences in total salivary DNA amplifying by PCR.[44] The several pathogens and microbiodata are also detected by this analysis.[45] The potential advantages of salivary analysis for the diagnosis of systemic disease suggest that further studies are warranted.[46]

Conclusion

The multifunctional roles of Saliva incur importance in today's life with its requisite advantages over other body fluids because salivary diagnostics offers an easy, inexpensive, painless, and stress free approach to disease detection. Whether saliva occurs in the large or small volume, recognition should be given to the many contributions it makes to the preservation and maintenance of better health from various infectious diseases/disorders. Early saliva diagnosis remains limited but promising, because of the improved efficiency of genomic and proteomic technologies which can prevent transmission of infections exhibit pandemic control.

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