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Saliva – A Potential Diagnostic Tool

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Abstract: *Saliva an important physiologic fluid, containing a highly complex mixture of substances is rapidly gaining popularity as a diagnostic tool. Saliva in the mouth forms a thin film, the velocity of which varies greatly at different sites and thus protects against dental caries, erosion, attrition, abrasion, periodontal diseases, candidiasis and the abrasive mucosal lesions. Early diagnosis and management of oral diseases reduces the severity and possible complications of the disease process. The use of saliva has provided a substantial addition to the diagnostic armamentarium as an investigative tool for disease processes and disorders. Its advantages as a diagnostic tool include its ease of procurement and the positive correlation between many parameters in the serum.*
Key Words: *saliva, serum, periodontal disease, dental caries, gingivitis.*

I. Introduction

Saliva is considered as the mirror of body health and is composed of variety of analytes from systemic sources that reach the oral cavity through various pathways. Saliva is a watery substance comprising of 99.5% water and 0.5% consists of electrolytes, mucus, glycoproteins, enzymes and antimicrobial compounds like IgA and lysozymes.Saliva can be considered as gland-specific saliva and whole saliva. [14]

Saliva is a hypotonic fluid composed of secretions from the major and minor salivary glands. The salivary glands are composed of specialized epithelial cells, and their structure can be divided into two specific regions: the acinar and ductal regions.

The acinar region is where fluid is generated and most of the protein synthesis and secretion takes place. Amino acids enter the acinar cells by means of active transport, and after intracellular protein synthesis, the majority of proteins are stored in storage granules that are released in response to secretory stimulation.The initial fluid is isotonic in nature and is derived from the local vasculature.

Ductal cells actively absorb most of the Na+ and Cl- ions from the primary salivary secretion and secrete small amounts of K+ and HCO3- and some proteins. The primary salivary secretion is thus modified, and the final salivary secretion as it enters the oral cavity is hypotonic

Early detection of disease plays a crucial role in successful therapy. Early diagnosis and management reduces the severity and possible complications of the disease process. A large number of diagnostic analytes have been shown to be present in saliva including steroid hormones and HIV antibodies.This paper highlights the applications of saliva in early diagnosis and assessment of various systemic and oral diseases.

GENERAL USES AND PROPERTIES OF SALIVA

The general uses and functions of saliva

- Lubrication
- Antimicrobial action
- Maintaining mucosa integrity
- Cleansing
- Buffer capacity and remineralisation
- Preparing food for swallowing
- Digestion
- Taste
- Phonation

The properties of saliva –

Healthy adult subjects normally produce 500-1500ml of saliva per day.

Normal values for stimulated flow rates are 1.0 - 3.0 ml/min. Unstimulated saliva flow rates in healthy individuals have found to be about 0.3 ml/min. [13]

Normal pH value of saliva is found to be 6.5-7.5

Saliva can be collected with or without stimulation. Stimulated saliva is collected by masticatory action. Unstimulated saliva is collected without exogenous gustatory, masticatory, or mechanical stimulation. Unstimulated salivary flow rate is most affected by the degree of hydration, but also by olfactory stimulation, exposure to light, body positioning, and seasonal and diurnal factors.

Compounds Expressed In Saliva

The various compounds of saliva are inorganic, organic non protein, protein/polypeptide, hormone and lipid molecules.[1]

- *INORGANIC COMPOUNDS*

  Whole saliva contains mainly water, strong and weak ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-,HPO_4^{2-}) which can generatebuffer capacity.Several factors may modify the salivary ionic concentration; furthermore, the composition of unstimulated saliva is different from stimulatedsaliva (which is more similar in composition to plasma).[6]

- *ORGANIC COMPOUNDS (NON-PROTEIN AND LIPIDS)*

Small amounts of organic non-protein compounds can bedetected in saliva. The organic compounds detected in saliva are Uric acid, bilirubin, creatinine, glucose, amino acids, lipids like cholesterol and mono/diglycerides.[12,16,23,26]

- *PROTEIN/POLYPEPTIDE COMPOUNDS*

Among the salivary proteins obtained by classical protein analysis methods, those deriving from salivary gland production can be recognized (of which the most abundant are amylase, PRPs secretory IgA (s-IgA) and carbonic anhydrase). Other proteins can be derived from plasma leakage (albumin, transferrin, IgG).The salivary immunoglobulins are mainly s-IgA (>85%) and are produced directly by the B lymphocytes present near the salivary glands. s-IgA is secreted in the interstitial fluid, taken up by the acinar and ductal cells of the salivary glands and subsequently secreted into the saliva.[17,19,20]

Further properties of human salivary proteins/peptides are:

1. Inhibition of calcium precipitation [32]
2. Taste perception [32]
3. Digestion [32]
4. Inhibition of proteinase [32]
5. Other functions: transcription, cell proliferation, signal transduction, chemotaxis and cell motility [32]

TABLE 1

SALIVARY PROTIENS:
[3,11,18,27,31]

TOTAL PROTEINS	CONCENTRATIONS	CLINICAL SIGNIFICANCE
α-Amylase	3257±1682 U/ml 1080.0±135.6 IU/l 476±191 μg/ml	Starch digestion
Albumin	0.2±0.1 mg/ml 0.8–192.0 mg/dl	Maintains blood volume
Cystatins Group	14.3 kDa form 58±25 μg/ml 14.2 kDa form91±46 μg/ml	Antimicrobial action
Hystatin	1190±313 μg/ml	Antifungal
Secretory-IgA	124.3–335.3 μg/ml	Antimicrobial
Lactoferrin	3.7±2.5 μg/ml	Antimicrobial
Lysozyme	3.5–92.0 μg/ml 21.8±2.5 mg/dl 59.7–1062.3 μg/ml	Antimicrobial
Transferrin	0.58±0.20 mg/dl	Iron transport and storage

- HORMONES

Hormones are commonly measured in plasma. They are steroids, non-steroids, peptide and protein hormones, can be detected in the oral fluid. Steroid detection is perhaps the most interesting applicationin salivary hormonal studies. The most commonly assayed biomarkers in saliva are cortisol, testosterone, dehydroepiandrosterone (DHEA), 17-hydroxyprogesterone, progesterone and aldosterone.Salivary cortisol measurement is today a widely accepted alternative to the determination in plasma or serum: since the adrenal cortex is responsive to stress, venipuncture for blood collection can lead to an iatrogenic increase of plasma glucocorticoid levels. In saliva other protein hormones which are also detected are prolactin, insulin-like growth factor I (IGF-I) and melatonin.[21,28,29, 40]

TABLE2
SALIVARY STEROID HORMONES:
[9,10,24,33]

COMPOUNDS	*CONCENTRATIONS & REFERENCES*	*CLINICAL SIGNIFICANCE*
Cortisol	Immediately after awakening: 3.6-35.1nmol/l	Treat adrenal insufficiency
Testosterone	140.30±154.15 pg/ml	Development of male reproductive tissue
DHEA-s	291.21±294.81 pg/ml	Fights aging
Progesterone	Luteal phase: 436±34 pmol/l Follicular phase: 22.1±2.7 pmol/l	Helps in embryogenesis
Estradiol	Luteal phase: 20.6±0.4 pmol/l	Reduce symptoms of menopause
Aldosterone	138–475 pmol/l	Regulates blood pressure

WHEN CAN SALIVA BE AN ALTERNATIVE TO PLASMA AND SERUM

The salivary glands, transfer mechanisms include intracellularand extracellular routes. The most commonintracellular route is passive diffusion.Ultrafiltration, which occurs through thetight junctions between the cells, is the mostcommon extracellular which must cross five barriers: the capillary wall, interstitial space, basalcell membrane of the acinus cell or duct cell, cytoplasm of the acinus or duct cell, and theluminal cell membrane for serum molecule to reach saliva. Serum constituents are also foundin whole saliva as a result of GCF outflow.Depending on the degree of inflammation in the gingiva; GCF is either a serum transudateor, more commonly, an inflammatory exudate that contains serum constituents. [25, 5,6,26,30]

SALIVA AS A DIANOSTIC TOOL

Saliva in diagnosis of diseases in general medicine:

- ***SYSTEMIC DISEASES***

1. HEREDITARY DISEASES

Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults, which is considered a generalized exocrinepathy. The elevated calcium and phosphate levels in the saliva of children diagnosed with CF.The submandibular saliva of CF patients contain more elevated levels of lipid, phospholipids, glycolipids, electrolytes (sodium, chloride, calcium, and phosphorus), urea and uric acid, and total protein.Saliva from CF patients was found to contain an unusual form of epidermal growth factor (EGF) and elevated levels of prostaglandins E2 (PGE2) were detected in the saliva of CF patients. [36, 37]

21-Hydroxylase deficiency is an inherited disorder of steroidogenesis which leads to congenital adrenal hyperplasia. In non-classic 21-hydroxylase deficiency, a partial deficiency of the enzyme is present. Salivary levels of 17-hydroxyprogesterone (17-OHP) were reported to be an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency, since the salivary levels accurately reflected serum levels of 17-OHP. [41, 42]

2. AUTOIMMUNE DISEASES- SJOGREN'S SYNDROME

Sjögren's syndrome (SS) is an autoimmune disorder. Sialochemistry may also be used to assist in the diagnosis of SS. Consistent finding is increased concentrations of sodium and chloride. This increase is evident in both whole and gland specific saliva. Elevated levels of IgA, IgG, lactoferrin, and albumin, and a decreasedconcentration of phosphate were reported in saliva of patients with SS. Other salivary changes associated with SS include an elevated concentration of b2 microglobulin, although differences exist between patients. In addition, elevated lipid levels and increased concentrations of cystatin C and cystatin S have been observed. Increased salivary concentrationsof inflammatory mediators—i.e., eicosanoids, PGE2, thromboxane B2, and interleukin-6—have been reported. Elevated levels of salivary soluble interleukin-2 receptor were also found in SS patients. Elevated levels of salivary kallikrein, IgA and IgG antibody have been found in association with SS. [35, 47]

3. MALIGNANCY

Salivary analysis may aid in the early detection of certain malignant tumours. Elevated levels of salivary defensin-1 were found to be indicative of the presence of oral Squamous Cell Carcinoma.Elevated levels of cancer antigen 15-3 (CA15-3) were found in the saliva of women diagnosed with breast carcinoma, as compared with patients with benign lesions and healthy controls.CA 125 is a tumour marker for epithelial ovarian cancer.Hence saliva can be potentially used in screening of malignant diseases.[8, 48, 50]

4. INFECTIOUS DISEASES

Helicobacter pylori infection is associated with peptic ulcer disease and chronic gastritis. Infection with this bacterium stimulates the production of specific IgG antibody. Testing for salivary antibodies against H. pylori yielded sensitivity of 85%, specificity which is comparable to serum IgG antibody.[34, 46]

5. VIRAL DISEASES

Saliva was found to be a useful alternative to serum for the diagnosis of viral hepatitis. Acute hepatitis A (HAV) and hepatitis B (HBV) were diagnosed based on the presence of IgM antibodies in saliva. Saliva has also been used for screening for hepatitis B surface antigen (HbsAg).Saliva may also be used for determining immunization and detecting infection with measles, mumps, and rubella.For newborn infants, the salivary IgA response was found to be a better marker of rotavirus (RV) infection than the serum antibody response.The shedding of herpesviruses (human herpesvirus –8, cytomegalovirus, and Epstein-Barr virus) in saliva of infected patients have been detected. Saliva is also used for the early detection of HSV-1 reactivation in patients with Bell's palsy. The shed HSV-1 virus was detected in 50% of patients with Bell's palsy.Salivary levels of anti-dengue IgM and IgG has been demonstrated in the diagnosis of primary and secondary infection, and salivary levels of IgG proved useful in differentiating between primary and secondary infections.[34]

- ***HIV :***

Studies have demonstrated that the diagnosis of infection with the human immunodeficiency virus (HIV) based on specific antibody in saliva is equivalent to serum in accuracy, and therefore applicable for both clinical use and epidemiological surveillance.Antibody to HIV in whole saliva of infected individuals, which was detected by ELISA and Western blot assay, correlated with serum antibody levels.Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may, therefore, be a prognostic indicator for the progression of HIV infection.Saliva can be collected non-invasively, which eliminates the risk of infection for the health care worker who collects the blood sample.Saliva collection also simplifies the diagnostic process in special populations in whom blood drawing is difficult, i.e., individuals with compromised venous access (e.g., injecting drug users), patients with haemophilia, and children.Several salivary and oral fluid tests have been developed for HIV diagnosis. Orasure is a testing system that is commercially available in the United States and can be used for the diagnosis of HIV. The test relies on the collection of an oral mucosal transudate (and therefore IgG antibody). IgG antibody to the virus is the predominant type of anti-HIV immunoglobulin.In conclusion, collection and analysis of saliva offer a simple, safe, well-tolerated, and accurate method for the diagnosis of HIV infection.[7]

6. DRUG MONITORING

The Therapeutic drugs monitoring in saliva are antipyrine, caffeine, carbamazepine, cisplatin, cyclosporine, diazepam, digoxin, ethosuximide, irinotecan, lithium, Methadone, Metoprolol , Oxprenolol, Paracetamol, Phenytoin, Primidone, Procainamide, Quinine, Sulfanilamide, Theophylline, Tolbutamide. The abusive drugs monitoring in saliva are Amphetamines, Barbiturates, Benzodiazepines, Cocaine, Ethanol, Marijuana, Nicotine, Opioids and Phencyclidine. [38]

7. MONITORING OF HORMONE LEVELS

Saliva can be analyzed as part of the evaluation of endocrine function. Salivary cortisol levels demonstrate excellent correlation with free serum cortisol levels.Salivary cortisol levels were found to be useful in identifying patients with Cushing's syndrome and Addison's disease.Salivary aldosterone levels demonstrated a high correlation with serum aldosterone levels, and increased aldosterone levels were found in both the serum and saliva of patients with primary aldosteronism. Testosterone and dehydroepiandrosterone have also been identified in saliva.Salivary testosterone levels were detected in an additional study which proposed the use of salivary testosterone levels for the assessment of testicular function. Estradiol can be detected in saliva in concentrations that are only 1-2% of serum concentrations. These concentrations are similar to the serum concentrations of free estradiol, which can diffuse into saliva. Decreased salivary estradiol was suggested as a marker of foetal growth retardation.Salivary progesterone levels can be useful for the prediction of ovulation, demonstrating a correlation of 0.75 with serum progesterone levels, and salivary estradiol and progesterone levels can be used for the evaluation of ovarian function.[4]

8. SALIVA AS A DIAGNOSTIC FLUID IN HALITOSIS

One of the most frequent complaints of dental patient’s concerns offensive breath. Halitosis or offensive breath may arise due to various local and systemic disorders like the teeth, gums, soft structures of the oral cavity, nasopharyngeal region, digestive tract, bronchopulmonary area and genital-urinary region. It is important

to have the ability of detecting abnormal shifts, both acid and alkaline, from the normal saliva pH of 6.9. An abnormal shift to the acid side may indicate the presence or atleast the susceptibility to dental decay and the more alkaline conditions point to calculus formation and the resultant likelihood of periodontal diseases associated there with. Another little known situation that can contribute to halitosis is xerostomia (dry mouth). When our mouth is dry, saliva production decreases, leaving the mouth's natural ability to clean itself impaired. Saliva is the mouth's natural mouthwash, which contains properties that reduces bacteria in the mouth.

9. SALIVA AS A DIAGNOSTIC FLUID IN PERIODONTAL DISEASES

Proposed salivary diagnostic markers for periodontal diseases have included serum and salivary molecules such as immunoglobulin, enzymes constituents of gingival crevicular fluid, bacterial components or products, volatile compounds, and phenotypic markers, such as epithelial keratins. The fluid mostly collected for salivary diagnostic purpose is expectorated whole saliva, a mix composed largely of the secretions from the major salivary glands along with the modest contributions from the minor salivary glands and gingival crevicular fluid.[13]

10. SALIVA AS A DIAGNOSTIC FLUID IN DENTAL CARIES

There is a reasonably evidence to state that salivary buffering capacity protects the tooth from dental caries.Since saliva provides a general protective effect, clinically significant decrease in salivary functions can be considered as an etiologic factor that contributes to the development of dental caries.In severe cases the unstimulated saliva results will also be low, giving a clear indication of increased levels of dental caries risk. If only conventional dental treatment is done in these subjects without treating the defect in the salivary parameters, recurrence of dental caries occurs in a relatively short time.[15, 39]

- *Saliva based caries activity tests include Lactobaccilus colony count test, Snyder test, Reductase test, Buffer capacity test, Fosdick calcium dissolution test, S.Mutans adherence method and S.Mutans dip-slide test.*

11. SALIVA AS A DIAGNOSTIC FLUID IN ORAL CANCER

Tumour cells inhabit or produce biochemical substances referred to as tumour markers. These can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remain quiescent in the normal cells. The various tumour markers in oral cancer are protein markers like M2BP, MRP14, CD 59, prifilin 1 and catalase , Genomic markers like Dual specificity phosphatase 1, H3 histone family 3A, Interleukin-1β, Interleukin-8, Ornithine decarboxylase antizyme-1, S-100P and Spermidine/spermine N1-acetyl transferase and salivary microbiota.

Advances like proteomics which is used to detect a panel of proteins, transcriptomics for DNA and RNA and genomics for determination of genetic damage are helpful for characterization of disease states.With point of care diagnostics being the requirement of the day salivary analysis could become a routine procedure for cancer detection.[11,22]

II. Conclusions

The past few years have seen the development of salivary diagnostic tools to monitor various oral diseases ranging from periodontal diseases, dental caries to infections and autoimmune diseases. The saliva matrix is an upcoming area of research for basic and clinical application purposes, with considerable potential for growth and progress.It is also usable for quantitative measurements of several analytes, particularly when a stable correlation between plasmatic and salivary levels can be achieved. Consequently, we are likely to see the increased utilization of saliva as a diagnostic fluid. As a result, dentists will have greater involvement in the identification and monitoring of certain non-oral disorders.

In conclusion, saliva is a biological fluid that offers several opportunities in diagnosis, toxicology and in forensic science. Furthermore, many salivary proteins offer great potential inclinical and epidemiological research, in oral as well as ingeneral health studies.

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