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REVIEW

Diagnostic Applications of Saliva

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ABSTRACT

Saliva is an easily accessible fluid that is rich in various proteins and enzymes. In addition to this, many drugs are also secreted in the saliva. It offers several advantages over blood as it is collected non-invasively and with much ease. In this article, the authors review various diagnostic applications of saliva.

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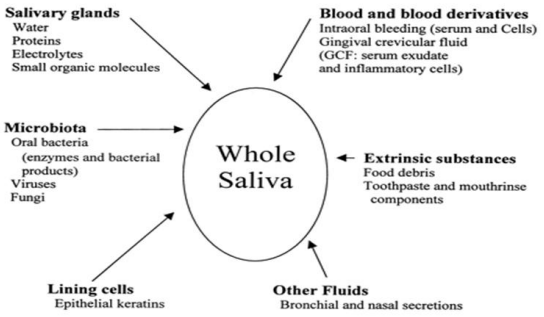
Introduction

This review examines the diagnostic applications of saliva for systemic diseases. As a diagnostic fluid, saliva offers distinctive advantages over serum, because it can be collected non-invasively by individuals with modest training and fewer complications occur as compared to those seen during blood collection. Furthermore, saliva may provide a cost-effective approach for the screening of large populations. Whole saliva is most frequently used for the diagnosis of systemic diseases, since it can be readily collected and as it contains the same constituents as that of serum. These constituents are derived from the local vasculature of the salivary glands and also reach the oral cavity via the flow of the gingival fluid [Table/Fig 1].



(Table/Fig 1) Component of Whole Saliva

Saliva has protective properties and contains a variety of antimicrobial constituents and growth factors. In addition, saliva has lubricating functions and aids in the digestion of food. The functions of saliva and the salivary constituents responsible for these functions are summarized in [Table/Fig 2].

(Table/Fig 2) The Major Functions of Saliva

Functions	Salivary Components Involved
(1) Protective functions	
Lubrication	Mucins, proline-rich glycoproteins, water
Antimicrobial	Amylase, complement, defensins, lysozyme, lactoferrin, lactoperoxidase, mucins, cystatins, histatins, proline-rich glycoproteins, secretory iga, secretory leukocyte protease inhibitor, statherin, thrombospondin
Growth factors	Epidermal growth factor (EGF), transforming growth factor-alpha (TGF-), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), insulin-like growth factor (IGF-I & IGF-II), nerve growth factor (NGF)
Mucosal integrity	Mucins, electrolytes, water
Lavage/cleansing	Water
Buffering	Bicarbonate, phosphate ions, proteins
Remineralization	Calcium, phosphate, statherin, anionic proline-rich proteins
(2) Food- and speech-related functions	
Food preparation	Water, mucins
Digestion	Amylases, lipase, ribonuclease, proteases, water, mucins
Taste	Water, gustin
Speech	Water, mucins

Saliva can be collected either by a draining method in which saliva is allowed to drip off the lower lip or by the spitting method in which the subject spits saliva into a test tube [1].

Traditionally, saliva tests were used to detect hepatitis, tuberculosis or strep throat. Today, technology uses highly sensitive methods of detection that have turned saliva into a tool for the diagnosis of many conditions.

Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, endocrine disorders, as well as for the assessment of the therapeutic levels of drugs and for the monitoring of illicit drug use.

Systemic Diseases (Hereditary, Autoimmune, Malignancy)

Hereditary Diseases

Cystic fibrosis (CF) is a genetically transmitted disease of children and young adults, which is considered as a generalized exocrinopathy. Elevated levels of calcium and proteins in submandibular saliva from CF patients were found and resulted in a calcium-protein aggregation which caused turbidity of saliva [2].The submandibular saliva of CF patients was also found to contain more lipid than the saliva of non-affected individuals and the levels of neutral lipids, phospholipids, and glycolipids were elevated [3]. Elevations in electrolytes (sodium, chloride, calcium, and phosphorus), urea and uric acid and total protein were observed in the submandibuar saliva of CF patients [4].

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. Early morning 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 the serum levels of 17-OHP [5].

A saliva-based miniature system can provide a powerful platform for rapid DNA extraction and for the detection of genetic diseases. The mutated alpha-globin gene which is associated with alpha-thalassemia-1 can be detected in saliva by several critical modules, including a genomic DNA (gDNA) extraction module, a polymerase chain reaction (PCR) module and an external optical detection module, all within less than 1 hr [6].

Autoimmune Disease

Sjögren's syndrome (SS) is an autoimmune exocrinopathy of unknown aetiology. SS is characterized by the presence of a lymphocytic infiltrate (predominantly $CD4^+$ T-cells) in the salivary gland parenchyma [7]. A low flow rate and an abnormally low stimulated flow rate of whole saliva are also indicators of SS. Autoantibodies, especially of the IgA class, can be synthesized in salivary glands and can be detected in the saliva of SS patients prior to their detection in the serum. In addition to IgA, saliva has also been reported to contain IgG autoantiboiesd [8]. SS anti-La antibodies were primarily found in the saliva of patients whose resting and stimulated whole saliva flow rates were abnormally low [9]. The expression of human beta-defensins 1 and 2 are decreased in salivary glands which are affected by Sjögren's syndrome in comparison with the human beta-

defensin expression patterns in the salivary glands from normal subjects [10].

Caeliac disease (CD) is a lifelong immune-mediated disorder which is caused by the ingestion of wheat gluten in genetically susceptible persons. Gluten stimulates naive lymphocytes directly in the oral cavity, which would have important implications for the understanding, diagnosis, and the management of CD by measuring CD-associated antibodies in saliva as a screening test [11].

Malignancy

A number of recent studies have focused on oncogenic marker detection and its monitoring in saliva. The latest clinical and laboratory findings on diagnostic markers of oropharyngeal carcinoma in oral fluid could be the beginning of their wider use as a diagnostic medium. Oral fluid can also be also used to diagnose other malignancies such as breast cancer, which was one of the first malignant tumours to be detected using genetic protein biomarkers [12].

Patients of head and neck carcinomas show reduced IgA levels in saliva but not in the serum. Causes associated with decreased salivary IgA levels like malnutrition, stress and tobacco could be related to these findings [13].

Salivary analysis may aid in the early detection of certain malignant tumours. P53 is a tumour suppressor protein which is produced in cells; p53 antibodies can also be detected in the saliva of patients who are diagnosed with oral squamous cell carcinoma (SCC) and can thus assist in the early detection of and screening for this tumour [14]. Autoantibodies against p53 proteins (p53 abs) can be detected in the serum, ascites, saliva and pleural effusions of various cancer patients [15]. Elevated levels of salivary defensin-1 were found to be indicative of the presence of oral SCC [16].

Elevated levels of recognized tumour markers c-erb-2 and cancer antigen 15-3 (CA15-3) were found in the saliva of women who were diagnosed with breast carcinoma, as compared to patients with benign lesions and healthy controls [17].

Elevated salivary levels of CA 125 were detected in patients with epithelial ovarian cancer. A positive correlation was found between salivary and serum levels of CA 125 [18].

A surface immobilized optical protein sensor has been utilized to detect Interleukin-8 (IL-8) protein, an oral cancer marker in saliva [19]. Neutrophil levels in saliva may also indicate successful bone marrow transplant in haematological malignancies [20].

Drug Monitoring

As with other body fluids (*i.e.*, serum, urine, and sweat), saliva has been proposed for the monitoring of the systemic levels of drugs [21]. The pka of the drug (the ph at which 50% of the drug molecules are ionized) and the ph gradient between plasma and saliva determine the concentration gradient on both sides of the membrane and influence the availability of a drug in saliva [22]. A significant correlation exists between salivary and serum lithium levels in patients receiving lithium therapy [23]. Saliva is also useful for the monitoring of anti-epileptic drugs like cyclosporine, theophylline, digoxin, Quinine, etc. [24],[25],[26].

Saliva can be an alternative specimen for the therapeutic monitoring of cyclosporine (CsA) in children and patients with difficult venous access. For a highly protein-bound drug such as CsA, saliva may also provide a practical approach for measuring the unbound concentration [27].

The trans-3'-hydroxycotinine(3HC)/cotinine (COT)3HC/COT ratio derived from nicotine which has either been administered as a probe drug or from tobacco use which is measured in either plasma or saliva, is highly correlated with the oral clearance of nicotine. This ratio appears to be a useful noninvasive marker for the rate of nicotine metabolism (which is important in studying nicotine addiction and smoking behaviour), as well as a general marker for measuring CYP2A6 activity (which is important in studying drug and toxin metabolism) [28].

Drug Abuse/Recreational Drugs can also be monitored eg Amphetamines, Ethano [29].

Monitoring of Hormone Levels

Saliva can be analyzed as a part of the evaluation of endocrine function. Salivary cortisol levels were found to be useful in identifying patients with Cushing's syndrome and Addison's disease and also for monitoring the hormone response to physical exercise and the effect of acceleration stress [30]. Evidence shows that salivary cortisol in evening samples or in those following dexamethasone suppression provides a reliable and effective screen for Cushing's syndrome [31]. Measurement of an elevated late-night salivary cortisol has greater than 90% sensitivity and specificity for the diagnosis of endogenous Cushing's syndrome. Late-night salivary cortisol measurements are also useful to monitor patients in remission and/or those having recurrence after pituitary surgery for Cushing's disease. [32].

Salivary aldosterone levels demonstrated a high correlation with serum aldosterone levels [33]. Increased aldosterone levels are found in both the serum and the saliva of patients with primary aldosteronism -Conn's syndrome. Measurement of aldosterone in saliva presents a useful and convenient method for application in multi-sampling studies [34].

Testosterone and dehydroepiandrosterone havealso been identified in saliva. Salivary testosterone levels are used for the assessment of testicular function [35]. Monitoring salivary testosterone levels may also be useful in behavioural studies of aggression, depression, abuse and violent and antisocial behaviour [36].

It is well known that late-onset hypogonadism in males can cause a variety of symptoms and that the differential diagnosis is relatively difficult, including psychological disorders, stress, and mood disturbances. The levels of serum cortisol can be measured to reflect a patient's level of stress. Simultaneous measurement of testosterone and cortisol levels in saliva is done by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [37].

Salivary estriol levels are used as a means for the assessment of foeto-placental function [38]. Decreased salivary estriol was suggested as a marker for foetal growth retardation [39].

Salivary progesterone levels can be useful for the prediction of ovulation, demonstrating a correlation of 0.75 with serum progesterone levels and salivary oestradiol and progesterone levels can be used for the evaluation of ovarian function. An increased salivary estriol-to-progesterone ratio may be a predictor of pre-term delivery [40].

To study pineal function in newborn infants, saliva collection using cotton buds and the measurement of melatonin in saliva offers a valid, non-invasive, pain-free and practical alternative to blood sampling and for the determination of serum melatonin [41].

Diagnosis of Oral Disease with Relevance for Systemic Diseases

Saliva can be used for the detection of oral candidiasis and salivary fungal counts may reflect mucosal colonization. Saliva may also be used for the monitoring of oral bacteria [42]. Bacteria (including anaerobic species) can survive in saliva and can utilize salivary constituents as growth media). Furthermore, increased numbers of *Streptococcus mutans* and Lactobacilli in saliva were associated with the prevalence and the presence of increased root caries [43]. Saliva can serve as a vector for bacterial transmission and also as a reservoir for bacterial colonization. Detection of certain bacterial species in saliva can reflect their presence in dental plaque and in periodontal pockets [44].

The collection and analysis of Gingivalcrevicular fluid (GCF) are noninvasive methods which are used for the evaluation of host response in periodontal disease. These analyses mainly focus on inflammatory markers such as prostaglandin E2, neutrophil elastase and beta-glucuronidase and on the marker of cellular necrosis-aspartat aminotransferase. Further, the analysis of inflammatory markers in GCF may assist in defining how certain systemic diseases

(e.g., diabetes mellitus) can modify periodontal disease and how peridontal disease can influence certain systemic disorders (atherosclerosis, preterm delivery, diabetes mellitus and some chronic respiratory diseases) [45].

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. Similarly, the analysis of saliva proved to be a highly sensitive and specific method for the diagnosis of viral hepatitis B and C. Saliva may also be used for determining immunization and also for detecting infections such as measles, mumps and rubella [46].

Antibody to HIV in whole saliva of infected individuals, which was detected by ELISA and Western blot assay, correlated with serum antibody levels [47].

Others

Rapid detection of *Plasmodium falciparum* histidine-rich protein II (*Pf*HRP II) antigen in saliva may be a useful non-invasive and cost-effective diagnostic technique for malaria [48].

Persons with periodontitis and type 2 diabetes have raised salivary Pro-calcitonin levels that reflect their degree of periodontitis activity and hyperglycaemia [49] . Salivary adiponectin may be used as a marker for the increased risk of non-insulin-dependent diabetes mellitus or for cardiovascular disease [50].

Conclusion

Saliva can be used as an alternative to serum as a biological fluid that can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. Whole saliva can be collected in a non-invasive manner by individuals with modest training, including patients. This facilitates the development and introduction of screening tests that can be performed by patients at home.

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