REVIEW ARTICLE

Saliva- A Diagnostic Fluid: A Review

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ABSTRACT

Saliva is emerging as a promising option for diagnosing and monitoring certain pathologies or dosages of drugs and medicines. Its advantages as a diagnostic tool includes its being easy to obtain and positive correlation between many parameters in serum and saliva. This is a review of current options for saliva as a diagnostic fluid.

KEYWORDS: Dental Caries, Diagnosis, HIV, Saliva, Salivary Biomarkers

INTRODUCTION

Saliva as a diagnostic specimen can give not only the same information as serum testing, but also additional or new information that cannot be obtained from serum.[1] Saliva in humans is a mouth fluid possessing several functions involved in oral health and homeostasis, with an active protective role in maintaining oral healthiness. Saliva helps bolus formation by moistening food, protects the oral mucosa against mechanical damage and plays a role in the preliminary digestion of food through the presence of α-amylase and other enzymes. It also facilitates taste perception, allowing soluble food-derived molecules to reach the gustative papillae and buffer the acid components of food with the bicarbonates (originating from salivary gland carbonic anhydrase).[2] The idea of using saliva in diagnostics was made in the second half of the 20th century. Its main advantage is easy and non - invasive sample taking compared to peripheral blood. As with the latter, salivary analysis has two main objectives: early detection of certain diseases and monitoring the disease course in conjunction with treatment. A third use is in the detection of addictive drugs.[3] Advances in the use of saliva as a diagnostic fluid have been affected by current technological developments: enzyme–linked fluorescence technique, Western blot assays, polymerase chain reaction (PCR).[4]

Saliva offers some distinctive advantages when used for diagnosis of disease. Whole saliva can be collected non-invasively, and by individuals with limited training, including the patient.[4] From a logistical perspective, the collection of saliva is safe, and simple, and it may be collected repeatedly without discomfort to the patient. Because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses, such

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as cancer, is of great interest for most salivary researchers.[5]

Saliva can be considered as gland-specific saliva and whole saliva. Evaluation of the secretions from the individual salivary glands is primarily useful for the detection of gland-specific pathology, *i.e.*, infection and obstruction. However, whole saliva is most frequently studied when salivary analysis is used for the evaluation of systemic disorders.[4]

Saliva/Oral Fluid Biomarkers	Possibilities for Use
DNA	Standard genotyping Bacterial infection Diagnosing carcinomas of the head and neck Forensics
RNA	Viral/bacterial identification Carcinomas of the head and neck
Proteins	Diagnosing periodontitis Diagnosing carcinomas of the head and neck Detecting dental cavities
Mucins/glycoproteins	Diagnosing carcinomas of the head and neck Detecting dental cavities
Immunoglobulins	Diagnosing viruses (HIV, hepatitis B and C)
Metabolites	Diagnosing periodontitis
Drugs and their metabolites	Monitoring drug abuse Detecting of drugs in the body
Viruses, bacteria	Epstein-Barr virus reactivation (mononucleosis)
Cellular material	Diagnosing carcinomas of the head and neck

Table No.1: Salivary Biomarkers with their possibility for use[3]

HORMONES AND SALIVARY LEVEL

Salivary steriod hormones have been used to assess ovarian function during in vitro fertilization, when monitoring hormone replacement therapy of post-menopausal women and the risk for preterm labour, to evaluate child health and development and to study mood and congnitive emotional behaviour.[6] Correlation between the salivary testosterone concentration and the serum free testosterone concentration is better than with total serum testosterone. Because most of the testosterone in the saliva is in the free form, Monitoring salivary testosterone levels may be useful to assess testicular function and behavioural studies of aggression, depression, abuse, and violent and antisocial behaviour.[4] Salivary cortisol levels were found to be useful in identifying patients with Cushing's syndrome, Addison's disease and the effect of stress.[7] Salivary aldosterone correlated significantly to plasma aldosterone levels, and increased aldosterone levels were found in both the serum and saliva of patients with primary aldosteronism (Conn's syndrome).[8]

SALIVA AS A DIAGNOSTIC TOOL

The salivary levels of pathogens such as porphyromonas gingivalis, streptococcus mutans, acidophilus can be utilized in risk assessments for periodontal disease and dental caries.[6] Counts of mutans streptococci and lactobacilli, Saliva secretion rate and buffering capacity have proven to be sensitive parameters in caries prediction models. High numbers of S.Mutans and Lactobacillus indicate a shift in oral microflora from healthy to more cariogenic. Diagnostic kits for S. mutans and Lactobacillus counting are widely used in dental practice and can be conducted without laboratory facilities.[4] The major salivary gland secretion mediators associated with periodontal diseases are shown in table II.[9]

Marker	Relationship with periodontal disease	Type of periodontal disease
Specific		
Immunoglobulins (IgA, IgM, IgG)	Interfere in adherence and bacterial metabolism / increased concentration in saliva of periodontal patients	Chronic and aggressive
Nonspecific		
Mucins	Interfere with the colonization of *Aggregatibacter actinomycetemcomitans*	Aggressive
Lysozyme	Regulates biofilm accumulation	Chronic
Lactoferrin	Inhibits microbial growth / increased correlation with *A. actinomycetemcomitans*	Aggressive
Histatin	Neutralizes lipopolysaccharide and enzymes known to affect the periodontium	Chronic and aggressive
Peroxidase	Interferes with biofilm accumulation / increased correlation with periodontal patients	Chronic
Systemic		
C-reactive protein	Increased concentration found in serum and saliva of periodontal patients	Chronic and aggressive

Table No.2: Major salivary gland secretion mediators associated with periodontal diseases[9]

VIRAL AND INFECTIOUS DISEASES

The antibody response to infection is the basis for many diagnostic tests in virology. Saliva contains immunoglobulins that originate from two sources: the salivary glands and serum. The predominant immunoglobulin in saliva is secretory IgA (sIgA),which is derived from plasma cells in the salivary glands, and constitutes the main specific immune defense mechanism in saliva. Although the minor salivary glands play an important role in sIgA-mediated immunity of the oral cavity, cells in the parotid and submandibular glands are responsible for the majority of the IgA found in saliva. In contrast, salivary IgM and IgG are primarily derived from serum via GCF, and are present in lower concentrations in saliva than is IgA. Antibodies against viruses and viral components can be detected in saliva and can aid in the diagnosis of acute viral infections, congenital infections, and reactivation of infection.[10]

Human immuno-deficiency virus infection is one of the best examples for utilizing saliva as a diagnostic aid. Several salivary and oral fluid tests have been developed for HIV diagnosis. Orasure is the only FDA- approved, commercially available testing system. It detects antibodies against the p24 antigen of HIV. The applicator swab is gently rubbed along the outer gumsand inserted into a vial containing the developer solution that detects the antibody to p24 antigen of HIV. [11] Saliva is also used for measurement as other viral pathogens such as hepatitis B, hepatitis C, cytomegalovirus, herpes simplex viruses and Epstein barr virus, it also received much attention in recent years for its potential role in the diagnosis of helicobactor pylori, the pathogen associated with peptic ulcer.[6] Saliva has also been used for screening for hepatitis B surface antigen (HbsAg) in epidemiological studies. Saliva may also be used for determining immunization and detecting infection with measles, mumps, and rubella. [12] Helicobacter pylori infection has been associated with peptic ulcer and chronic gastritis. Oral cavity may be the source of infection. There was considerable variation in the detection rate of H.pylori DNA in salivary samples. There is controversy whether saliva act as permanent reservoir for this bacterium and gastric and salivary specimen harbor identical or different strains. PCR cannot distinguish between living and dead organism.[13]

In the children infected with Shigella revealed higher titers of anti-Shiga toxin antibody in comparison with healthy controls.[14] Pneumococcal pneumonia the detection of pneumococcal C polysaccharide in saliva by ELISA may offer a valuable complement to conventional diagnostic methods for pneumococcal pneumonia. Quantitative measurement of pneumococcal capsular antigen in the saliva may be valuable in helping to make an aetiological diagnosis in children with pneumonia.[15]

ROLE IN PREMALIGNANT AND MALIGNANT DISORDERS

Numerous candidate proteins were discovered in saliva of patients with oral lesions, however, not all proteins have biomarker ability in diagnosing oral cancers. Bioinformatic analysis of exfoliated epithelial cells from subjects' saliva revealed increased myosin and actin abundance in those with malignant lesions as confirmed by western blotting. Salivary actin and myosin abundances distinguish oral lesion types with sensitivity and specificity rivaling other noninvasive oral cancer tests. These findings provided a promising starting point for

the development of noninvasive and inexpensive salivary tests to reliably detect oral cancer early. [16]

CA15-3, a high-molecular-mass mucin- like glycoprotein, is expressed at the luminal surface of most secretary epithelial. Of the tumor markers examined in breast cancer, it is the best and the most extensively used one as its expression greatly increases in most breast carcinomas. Hosseini et al showed in the study that levels of circulating CA15-3 correlated with tumor size, thus reflecting disease stage and indicated that the mean serum CA15-3 was significantly higher in untreated breast cancer group than in healthy women.[17] Tumour marker c-erbB-2 (erb) was found to be present in the saliva of breast cancer patients and absent in control subjects, possibly representing a more robust biomarker of breast cancer than CA15-3(35). EGF overexpression is thought to be linked to tumourigenesis. Salivary EGF was found to be significantly raised in women with active and nonactive breast cancer compared to healthy women. [18]

ROLE IN ANALYSIS OF HEREDITARY AND AUTOIMMUNE DISEASES

21-Hydroxylase deficiency is an inherited disorder of steroidogenesis which leads to congenital adrenal hyperplasia. Early morning salivary levels of 17-hydroxyprogesterone (17-OHP) determined by ELISA is an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency, since the salivary levels accurately reflected serum levels of 17-OHP. Sjögren's syndrome (SS) is an autoimmune exocrinopathy of unknown etiology. Serum chemistry can demonstrate polyclonal hypergammaglobulinemia and elevated levels of rheumatoid factor, antinuclear antibody, anti-SS-A, and anti-SS-B antibody. In addition, increased concentrations of sodium and chloride, IgA, IgG, lacoferrin, and albumin, and a decreased concentration of phosphate were reported in saliva of patients with SS. [4]

FORENSIC EVIDENCE

Saliva may be found on victims of several violent crimes. Saliva can potentially be recovered from bite marks, cigarette butts, postage stamps, envelopes and other objects. Stains of dried saliva are invisible, making its recognition and collection difficult. However, the presence of saliva can be confirmed by amylase assay. During the biting process, saliva is deposited on the skin or object surface in enough amount to allow typing of the deoxyribonucleic acid (DNA). 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. [19]

ROLE IN DRUG

It can be used to monitor a patient's compliance with insulin therapy, psychotherapy and anticancer medications. It can also detect the drug use, recreational drug, tobacco consumption and ethanol consumption. A number of medications can be easily monitored in saliva.[6] Salivary ethanol concentration may be used as an index of the blood ethanol concentration, provided that the salivary sample is obtained at least 20 min following ingestion. This will allow for absorption and distribution of alcohol, and prevent a falsely elevated reading due to the oral route of consumption. Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine (PCP), and opioids.[20]

Nicotine saliva can be used to monitor tobacco smoking and exposure to tobacco smoke. The major nicotine metabolite cotinine was

investigated as an indicator of exposure to tobacco smoking. Cotinine is tobacco-specific and has a relatively long half-life (17hours) compared with nicotine. Salivary cotinine levels were found to be indicative of active and passive smoking. Monitoring level of salivary cotinine has proven useful in monitoring compliance with smoking cessation programs.[21,2]

DRUG MONITORING IN SALIVA	
Antipyrine	Phencyclidin
Carbamazepine	Paracetamol
Caffeine	Phenytoin
Cisplatin	Primidone
Cyclosporine	Quinine
Diazepam	Procainamide
Digoxin	Sulfanilamide
Ethosuximide	Theophylline
Irinotecan	Tolbutamide
Lithium	Drug Abuse/Recreational Drugs
Methadone	Amphetamines
Metoprolol	Benzodiazepines
Oxprenolol	Barbiturates
Ethanol	Cocaine
Marijuana	Nicotine
Opioids	Phencyclidine

Table No.3: Drug monitoring in saliva [2]

CONCLUSION

Although blood is the gold standard for diagnosis of drugs and diseases, the success of saliva as a diagnostic media is guaranteed, particularly for substances that reflect or can be directly correlated with systemic analytes.

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