Saliva as Diagnostic Tool – A Review

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INTRODUCTION

The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. In recent years due to the invasive nature of blood sample collection and the hazards of cross infection; the need for finding other body fluids as an alternative to blood plasma or serum has immensely increased.

Saliva is the product of multiple salivary glands lying beneath the oral mucosa, which is a readily available specimen, that can be collected by noninvasive procedures. The multifarious components within saliva not only protect the integrity of the oral tissues, but also provide clues to local and systemic diseases and conditions.

Each day, the human salivary glands produce almost 600mL of serous and mucinous saliva containing minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immunoglobulins (e.g., secretory immunoglobulin A [sIgA]), mucins and other glycoproteins. It also contains hormones, drugs, antibodies and many analytes of interest for screening and diagnosis.

Saliva can be easily collected in remote sites by unskilled personnel, by spitting or with certain collection devices, is stable at ambient temperatures for several weeks. It provides a cost-effective approach for the screening of large populations. Diagnosis of disease via analysis of saliva is potentially valuable for children, elderly and medically compromised population, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Many of the hazards associated with blood collection do not apply to saliva. There is no need for sharps, which have the potential for cross contamination among patients when used improperly and present a danger to health care personnel. It is increasingly being investigated as an alternative to serum for diagnostic and epidemiological testing. This review examines the diagnostic application of saliva for various systemic diseases.

Sample collection processes

Saliva can be collected with or without stimulation. Stimulated saliva is collected by masticatory action (i.e., from a subject chewing on paraffin) or by gustatory stimulation (i.e., application of citric acid on the subject's tongue). Stimulation obviously affects the quantity of saliva; however, the concentrations of some constituents and the pH of the fluid are also affected. 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 factor.

The most common way to collect saliva is by direct spitting into a tube. There are few devices that filter the specimen, one method is by placing a small membrane sack in the mouth such as Saliva Sac and other methods use a tiny plastic tube containing cyclodextrin to bind the analyte such as Oral Diffusion Sink (ODS). Absorbent pads or balls are also used to collect saliva, especially for qualitative testing such as OraSure; Epitope, Beaverton, OR and Salivette. The absorbent pad is immersed in a small amount of fluid containing preservative to stabilize the specimen for several weeks. The ODS has the interesting property of being able to collect an average level over several hours. The device is suspended in the mouth by means of dental floss and saliva bathes it for the desired number of hours. The patient can sleep or do normal activities other than eating or drinking. The level obtained by extracting analyte from the device is the average over the time period.
AVAILABLE DIAGNOSTIC METHODS

Saliva dipstick method
Saliva test kits for collection, processing and analysis of the specimen are available. Immunochromatography test strips with a sample indicator are used for this purpose.

Culture and sensitivity
Saliva is collected on a swab, cultured for bacterial diagnosis and sensitivity is then checked against various antibiotics.

Fluorescent antibody method
Certain small molecular weight fluorescent compounds that absorb light (energy) of one wavelength and emit light of another wavelength can be conjugated to antibody molecules to detect antigens in tissue sections or solutions. When a given antigen is treated with a specific fluorescent antibody, the entire complex becomes visible, under a fluorescent microscope.

Radio immunoassay (RIA)
This technique is used for detection of trace amounts of hapten or antigens such as hormones. The procedure is based on the competition for the reactive sites on the specific antibody between known amounts of a highly radioactive antigen and unknown amount of the same but non-radioactive antigen. RIA is usually carried out in antigen excess.

Enzyme linked immunosorbent assay (ELISA)
The radioactive tag used in RIA can be replaced with certain enzymes. When this enzyme is linked to an antibody and used to detect other antibody or antigens, the assay is called ELISA. Assay depends upon the ability to attach an active enzyme (such as alkaline phosphatase or horse radish peroxidase) to an antibody molecule.

Polymerase chain reaction (PCR)
It is a technique for amplification of specific DNA sequences, by making multiple copies of initial template molecule and used in cases where the amount of DNA fails detection using conventional probes. For example when 1 out of 1000 cells are infected in HIV infection. The technique uses specific primers for each end of nucleotide sequences to be amplified. DNA polymerase plus highly labeled nucleotide sequences to be amplified is present; it can be copied many times over a period of several hours. It has a tremendous potential for speedy diagnosis of infectious diseases caused by viral or bacterial DNA and also in analysis of genetic diseases.

Blotting methods
Visualization of a specific DNA or RNA fragment among many thousands of contaminating molecules require the convergences of a number of techniques collectively called Blot transfer e.g. Southern blot (DNA), Northern blot (RNA), Western blot (proteins). The method is to transfer DNA (or RNA or proteins) from an agarose gel to a nitrocellulose filter on which DNA can be detected by a suitable probe. It hybridizes to a complementary fragment on the filter, which is then visualized following routine procedures of washing and developing.

Diagnostic value of saliva
The use of saliva as a diagnostic fluid has been successfully applied in diagnostics and for predicting populations at risk for a variety of conditions. Diagnostic biomarkers in saliva have been identified for diagnosis and monitoring caries, periodontitis, oral cancer, salivary gland diseases, endocrine diseases, systemic disorders, HIV, Hepatitis B.

Bacterial diseases
Helicobacter pylori
Helicobacter pylori infection is associated with peptic ulcer disease and chronic gastritis. Infection with this bacterium stimulates the production of specific IgG antibody. An ELISA test for the detection of IgG antibody in serum produced 97% sensitivity and 94% specificity in detection of the disease. In parallel, saliva samples were tested for the presence of H. pylori DNA by polymerase chain-reaction (PCR) assay, and sensitivity of 84% was reported. The results also indicated that H. pylori exists in higher prevalence in saliva than in feces, and the oral-oral route may be an important means of transmission. In another study, testing...
for salivary antibodies against *H. pylori* yielded sensitivity of 85%, specificity of 55%, positive predictive value of 45%, and negative predictive value of 90%.

**Viral diseases**

**HIV**

*Human immunodeficiency virus* HIV (HIV-1) is a human type C retrovirus and is closely related to HIV-2, which causes a similar disease primarily in West Africa. HIV-1 and 2 antibodies tested on normal and HIV samples by saliva dipstick, showed 99.5% and 94.7% sensitivity respectively when compared to a routine ELISA tests on serum samples. This confirms the potential for saliva testing in situation where it would be inconvenient to use blood. HIV antibodies were screened in paired samples of serum and saliva from sero-negative subjects with risk factors for HIV infection, symptomatic and asymptomatic seropositive patients and healthy blood donors as negative controls and the results confirmed by western blot, show 99% specificity and 99% sensitivity respectively. This method of saliva sampling for HIV antibody detection may be applicable in studies conducted with limited technical recourses or where blood sample collection is difficult. Like wise matched serum and saliva specimen for HIV were found to show 100 and 99.1% sensitivity and specificity respectively. The method is thus recommended for use in the field where there is lack of trained personals and laboratory support in surveillance studies and in community hospitals.

**Hepatitis**

*Hepatitis A* virus (HAV) is a member of single stranded RNA picorna virus family whereas B virus (HBV) is a DNA virus of hepadna virus family. Saliva may be used as an alternative to blood sera in diagnosis of hepatitis A, B and C in patients. Oral fluid samples were compared with serum samples as a specimen source for hepatitis A, B, and C virus markers. Oral fluid was obtained with a treated absorbent pad and tested by using existing commercial enzyme immunoassays with only minor modifications. Compared with serum sampling the sensitivity and specificity of oral sampling were 100% (51 of 51 samples) and 98% (46 of 47 samples) for hepatitis A virus immunoglobulin M, 100% (29 of 29 samples) and 100% (29 of 29 samples) for hepatitis B virus surface antigen, and 100% (13 of 13 samples) and 100% (13 of 13 samples) for hepatitis C virus antibody, respectively. The decline of hepatitis A virus immunoglobulin M in oral samples was parallel to, though somewhat more rapid than, that of hepatitis A virus immunoglobulin M in serum samples. It can be proposed that oral sampling represents a safer and more convenient procedure for reliable hepatitis virus testing than blood sampling and that it has wide application in patient and outbreak management.

In another study to assess the efficacy of oral fluid antibody testing for the detection of hepatitis C, paired serum and oral fluid collections were obtained from 216 subjects. A modification of the serum HCV ELISA assay was developed to improve test accuracy for an oral fluid substrate. Sensitivity was determined in 109 HCV serum ELISA-positive patients and specificity in 107 HCV serum ELISA-negative patients. RESULTS: Overall sensitivity of oral fluid collection and testing was 98.2%; specificity was 99.1%. These parameters did not seem to be altered by presence of concurrent hepatitis B infection, inflammatory state of the liver, or other factors. Oral fluid collection and HCV antibody testing by the modified ELISA method seems to be effective and efficient alternatives to venepuncture and serum HCV antibody testing. Their use may facilitate epidemiological surveys and evaluation of individual patients when blood collection is not feasible.

**Malignancy**

Salivary analysis may aid in the early detection of certain malignant tumors. p53 is a tumor suppressor protein which is produced in cells exposed to various types of DNA-damaging stress. Inactivation of this suppressor through mutations and gene deletion is considered a frequent occurrence in the development of human cancer. As a result, accumulation of inactive p53 protein is observed, which in turn may lead to the production of antibodies directed against this protein. These antibodies can be detected in sera of patients with different types of malignancies. p53 antibody can
also be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection of, and screening for, this tumor.

Defensins are peptides which possess antimicrobial and cytotoxic properties. They are found in the azurophil granules of polymorphonuclear leukocytes;23,24 Elevated levels of salivary defensin-1 were found to be indicative of the presence of oral SCC. Higher concentrations of salivary defensin-1 were detected in patients with oral SCC in comparison with the defensin-1 concentration in the saliva of patients with adenocarcinoma and in healthy controls. A high-positive correlation was observed between salivary defensin-1 levels and serum levels of SCC-related antigen25.

In a recent preliminary study, elevated levels of recognized tumor markers c-erbB-2 (erb) and 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. However, while low levels of CA15-3 were also detected in the saliva and serum of healthy individuals, erb was not detected in healthy subjects and thus appears to hold greater promise for the early screening and detection of breast cancer26.

CA 125 is a tumor marker for epithelial ovarian cancer. Elevated salivary levels of CA 125 were detected in patients with epithelial ovarian cancer as compared with patients with benign pelvic masses and healthy controls. A positive correlation was found between salivary and serum levels of CA 125. A further analysis of this relationship revealed that saliva demonstrated a somewhat lower sensitivity than serum (81.3% vs. 93.8%, respectively); however, the specificity and positive predictive value were higher for saliva vs. serum (88.0% vs. 59.8% and 54.2% vs. 28.8%, respectively27.

Tumor markers that can be identified in saliva may be potentially useful for screening for malignant diseases. Salivary diagnosis may be part of a comprehensive diagnostic panel that will provide improved sensitivity and specificity in the detection of malignant diseases and will assist in monitoring the efficacy of treatment. Additional studies are certainly required to determine which salivary markers can be used for these diagnostic purposes, and to determine their diagnostic value in comparison with other, more established, diagnostic tests1.

The monitoring of hormone levels

Saliva can be analyzed as part of the evaluation of endocrine function. Salivary steroids are thought to reflect the concentration of unbound serum steroids. In this respect the salivary concentration may be a better measure of the exposure of target organs to the steroids than the serum concentration are28.

Thyrotropin receptor antibody was measured with a radio receptor assay in parotid saliva and in the serum in the same patients. There was good correlation between salivary and serum level, and in 3 pathologic studies TR Ab levels were higher in saliva than in serum (Graves disease, Hashimoto's thyroiditis)29. Detection of 17-beta estradiol in saliva is useful for fertility monitoring and management by direct colorimetric antibody enzyme immunoassay30. Progesterone in saliva is tested by simple, direct and very sensitive radio immunoassay (RIA). Collection of saliva is more convenient and less invasive technique, for frequent sample collection than phlebotomy and is useful for monitoring ovulation and assessment of luteal function in women clinically31. Fluorescence immunoassay for salivary testosterone provides a useful tool for monitoring androgen status in men and women and is well suited for follow up of testosterone replacement therapy on an out patients basis32. Salivary samples can be used for determination of Erythropoietin (EPO) in adults, full term and premature infants, as an alternative method to blood sampling. ELISA measured serum and saliva EPO concentration. Significant correlation was observed between serum and salivary concentrations in each group.

Insulin can be detected in saliva, and salivary insulin levels have been evaluated as a means of monitoring serum insulin levels. A positive correlation between saliva and serum insulin levels following a glucose tolerance test was reported for healthy subjects (r = 0.52), non-insulin-dependent diabetic patients (r = 0.50), and obese non-diabetic
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patients (r = 0.69)\textsuperscript{33}. Additional work by the same authors utilizing similar methods reported a better correlation between salivary and serum insulin levels in 93 healthy subjects (r = 0.75 in males and r = 0.72 in females)\textsuperscript{34}. As assessed by radioimmunoassay, a glucose tolerance test performed on nine healthy patients produced a positive correlation between salivary and serum insulin levels (r = 0.74). Salivary insulin levels reached maximal values approximately 30 minutes after the serum levels (90 min vs. 60 min)\textsuperscript{35} Other investigators also reported a similarly high correlation between salivary and serum insulin levels in healthy individuals and insulin-dependent diabetic patients (0.81 and 0.91, respectively), but proposed that the use of salivary insulin levels for the evaluation of serum insulin levels could be misleading, since significant discrepancies between salivary and serum insulin levels were detected for several individuals\textsuperscript{36}. Additional studies are required to determine if salivary insulin levels should be used for the evaluation of serum insulin levels.

DISCUSSION

The hazards of blood collection are increasing day by day. Repeated use of disposable syringes and improper blood drawing techniques are leading to increase in incidence of various diseases like hepatitis and AIDS. In view of this, the need for finding other body fluids as an alternative to blood plasma or serum has immensely increased. Biochemical study of saliva has an important role in diagnosing diseases. There seems a good correlation between the level of the constituents in saliva and blood plasma so determination of constituent level in saliva can be used for diagnostic and/or monitoring purposes. Further the main advantage of saliva analysis resided in stress free and harmless collection of saliva in comparison with blood withdrawing. The advancement in the diagnostic procedure such as ELISA, polymerase chain reaction and Western blot methods has brought a revolution in medical diagnosis. Body fluids other than serum are being used in the diagnosis of diseases, which were previously not helpful, because these procedures allow accessing small concentrations of active substances in these body fluids. Saliva is one of them. However the use of saliva for diagnostic purpose is still at its beginning. The saliva analysis has a very prospective chance to substitute or alternate the biochemical analysis of blood plasma.

Saliva meets a demand of an inexpensive, noninvasive and accessible fluid to act as an ideal diagnostic medium. Specific and informative biomarkers in saliva are greatly needed to serve for screening, diagnosing diseases and monitoring human health.

REFERENCES


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