Short Communication

DIAGNOSIS IN A SPIT : A SHORT REVIEW

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ABSTRACT

Salivary diagnostics is a cost effective, minimally invasive procedure. This bio-fluid can be easily collected, transported and stored. There are a variety of conditions where saliva can be used as a diagnostic tool such as measurement of stress, enzyme levels, developmental disease biomarkers and cancer mutations. A number of studies have focused their attention on the assessment and monitoring of oncologic biomarkers in saliva. Modern techniques, chemicals and various equipment have made laboratory investigations of saliva easy and precise. Its clinical applications and analysis has made salivary diagnostics a useful tool in the field of Medicine and Dentistry. The methodology for diagnosis of oral and systemic diseases has been a subject of study by several researchers with the aim of increasing its use as a diagnostic tool.

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INTRODUCTION

Saliva is a unique bio-fluid that has varied amount of proteins, polypeptides, nucleic acids, electrolytes, and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 7.2–7.4 (1). Saliva as a bio-fluid has been a subject of research for many years but interest in its biochemical and physicochemical properties gained impetus when researchers found that it is filled with hundreds of components that may serve to detect systemic disease or evidence of exposure to various harmful substances, as well as provide biomarkers of health and disease status (2,3). The collection and analysis of saliva is non-invasive and cost effective. An understanding of the salivation mechanism coupled with advanced techniques and equipment can help in the confirmation of the molecular basis of many disorders. This in turn will open up the possibility of "point of care" diagnostics using the oral bio-fluid (4).

SALIVA VS SERUM

Saliva has been identified to be functionally equivalent to serum. It reflects the physiological state of the body, including hormonal, emotional, nutritional, and metabolic variations (5). It is one of the easiest body fluids to collect as it is a simple, non-invasive chair side procedure and does not require any special equipment. It is cost effective and can be used as a diagnostic tool for mass screening purposes. There is a facility for repeated sampling in a short interval of time. The sampling procedure is safe for the operator and the patient. Saliva

has thus been found to be effective as a diagnostic tool (6, 7).The linkage of saliva with traditional biochemical parameters which appear in the serum makes it an interesting diagnostic tool (8).

METHOD OF COLLECTION

Saliva can be collected either in stimulated or un-stimulated conditions. Un-stimulated saliva is collected by allowing saliva to collect in the mouth and then it is either drained in a wide bore sterile vessel or collected by using a cotton swab in the mouth or by using suction methods. Stimulated whole saliva is collected by asking the patient to chew paraffin wax. The same can also be collected by applying acetic acid in the mouth (9). The disadvantage of using stimulated saliva is that the bio fluid collected may not only be watery in secretion but will also have altered Ph. However, it is the method of choice when there is a decrease in salivary secretion. Recently new methods of collection of saliva have been introduced (10). Oragene is a commonly used sophisticated technique where preservation buffers are used to protect the sample until processing and extraction take place. Other methods of saliva expectoration include Saligene, Oracol, and Verofy.

SALIVA AS A DIAGNOSTIC TOOL

Role of saliva in oral diseases such as dental caries and periodontitis

There are a number of antimicrobial agents present in saliva which have been further divided into immune and non-immune factors. These factors affect the

micro-organisms alone or in combination (11). There are different saliva based tests such as lactobacillus colony count test, which estimates the number of acidogenic and aciduric bacteria in the patient's saliva. Synder test and Reductase test are other tests which measure the rapidity of acid formation and measures the the colour change of diazoresorcinol on reduction by the salivary flora respectively.

Saliva based tests for periodontal diseases
Pyridinoline crosslinks, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP),represent a potentially valuable diagnostic aid for periodontal disease (12)

Diagnosis of infections
Oral fluids contain various biomolecules of which IgG is derived from the gingival crevicular fluid and the mucosal transudate. IgG levels in saliva are the antibodies used in the screening of viral infections and immunization. Viral lesions are associated with the shedding of the virus into the saliva (13). The salivary antibodies of human immunodeficiency virus (HIV),hepatitis C virus (HCV), hepatitis A virus (HAV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and rubella virus have a considerable correlation with those of the serum values. Salivary antibodies have also been found positive following immunization against poliovirus, rotavirus, and HIV (14).

Diagnosis of tumor
Saliva is an emerging diagnostic method in the diagnosis and prognosis of malignancies. Tumour markers like c-erb B2, p53, and CA125 which are used in screening and diagnosis of several malignancies. The genetic analysis and their expression levels can be determined by salivary RNA transcript with microarray analysis (15). Application of salivary diagnostics also includes the detection of ovarian cancers, breast cancers using tumour markers (16). p53 antibody can 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. It has been suggested that salivary nitrate, nitrite, and Nitrosamine may be related to the development of Oral and Gastric Cancer (17).

Monitoring of drug levels
Saliva serves as a diagnostic tool in determination of various pharmaceutical drugs like lithium and digoxine phenol barbital which have a narrow therapeutic index. Further, alcohol, amphetamines, barbiturates, and opioids, which serve as commonly used abused drugs, can also be monitored using saliva (18,19).

An assessment of hormone levels
Saliva serves as a tool in determination of protein polypeptide hormones and non-peptide hormones (20).

Hormones whose salivary levels reflect serum levels are Cortisol, Progesterone, Aldosterone, I7 beta-Estradiol, Dehydroepiandrosterone, Testosterone,5alpha-Dihydrosterone, Estriol, Estrone, Insulin, Melatonin (21).

Sialochemistry
Heavy metals such as lead and cadmium are important occupational toxins and can also be found in saliva**.** The presence of these heavy metals in saliva occurs by diffusion (18)

Diagnosis of autoimmune disorders
Sjogren's syndrome is an autoimmune endocrinopathy characterized by xerophthalmia, xerostomia, and keratoconjunctivitis. IgA secreted by the salivary gland serves as a marker in Sjogren's syndrome patients (22)

Saliva and wound healing
Saliva helps in wound healing apart from its role in preventing wound infections. The EGF present in saliva has angiogenic and proliferative effects which enhances the wound healing (23). Saliva also contains clotting factors like IXa, VIII, and XI. Increased level of salivary kallikrein has a major role in vasodilatation around mucosal injuries to facilitate defence and healing of injured areas (24).

Role of saliva in other systemic disorders
Acute stress conditions also lead to significant salivary changes with a prominent decrease in secretary IgA and increase in salivary amylase and molecular chaperoneHsp70. There is also a prompt change in the bacterial adherence to the mucins. Saliva has been used to assess the salivary creatinine levels in diagnosis and monitoring of the kidney failure (25). The presence of cardiac enzymes in saliva helps in obtaining promising results in the identification of acute myocardial infarction (26). Cystic fibrosis can be detected by measuring the lipid levels of submandibular saliva which is considerably raised leading to increased calculus formation. Abnormally elevated prostaglandins E2 and poor biologic activities of EGF are also key features of cystic fibrosis (25).

LIQUID BIOPSY AND CIRCULATING CELLS AND DNA
Saliva has been useful as a liquid biopsy tool in point of care diagnostics as it is a convenient method of diagnosing and identifying cancer types from biofluids, instead of using conventional biopsy of tissue sampleswhich is an invasive procedure. Circulating tumor DNA (ctDNA) is a constituent in saliva, which can be extracted or detected using specialized techniques. As opposed to the various purification techniques that require high sample volumes and specialized equipment, ctDNA targets can simply be

isolated through traditional DNA extraction methods (27). On a clinical level, it should also be noted that ctDNA may also aid in other molecular diagnostics besides cancer. Circulating cell-free DNA can furthermore indicate several conditions such as autoimmune diseases, stroke, sepsis, trauma, and myocardial infarction (28, 29). Both circulating tumor cells and ctDNA targets for cancer profiling offer various advantages and disadvantages, and while there is no definitive answer regarding the future of liquid biopsy diagnostics, in general, it can be stated that ctDNA has occupied a dominant position in recent years since it is simpler to work with and does not require the usage of specific extraction methods such as beads or microfluidics to achieve separation from a biofluid sample (30).

Appearance of tumor markers in saliva

Although it is inevitable that the markers of oral cancer will be reflected in the saliva, the relationship between systemic disease/ cancer and appearance of biomarkers in saliva is yet unclear. Several intracellular and extracellular pathways enable the biomolecules to move from blood capillaries to the saliva (31) Many of the biomolecules enter into saliva from the blood by passing through the spaces between cells by transcellular (passive intracellular diffusion and active transport) or paracellular routes (extracellular ultrafiltration). These markers may be carried by the local capillaries of the salivary glands to the oral cavity through the flow of gingival crevicular fluid (GCF) (17). In an experiment conducted on tumor bearing mice an altered salivary protein pattern was noted which confirmed the speculation of the researchers that the tumors secrete mediators which may affect the activity of transcription factor in salivary glands, thereby inducing either an up or down-regulation of protein transcript levels in saliva (32).

NEWER DIAGNOSTIC TECHNIQUES

MyPerioID and MyPerioPath are DNA based saliva tests to determine the type and concentration of bacteria that cause periodontal disease. My PerioID test also determines the genetic susceptibility to periodontal disease and identifies patients of risk (33). Oral fluid nano-sensor test is a micro electromechanical system that is capable of real time, ultrasensitive, ultra specific detection of salivary protein and RNA biomarkers (34). This envisioned product has a great value in multiplex detection of salivary biomarkers for oral cancer patients. It also analyses saliva for the presence of salivary mRNA biomarkers (SAT,ODZ, IL-8, and IL-1b) and two salivary proteomic biomarkers(thioredoxin and IL-8).

Ora Quick is an antibody test which detects the HIV1 and HIV2 in the saliva, serum, and plasma and is a quick chair side test which provides results in 20 minutes. Integrated microfluidic platform for oral diagnostics (IMPOD) is a point of care diagnostic test which enables rapid quantification of a biomarker associated with the oral disease from the saliva. It is one of the best methods for detection of MMP8 and other biomarkers in small concentrations of saliva (35). Integrating the new salivary diagnostic methods into clinical practice will aid the clinicians in making health related decisions for the patients.

CONCLUSION

Saliva is a complex and dynamic biological fluid which contains a variety of compounds. Salivary diagnostics is an emerging field of research. Biomarkers found in saliva comes from the the gingival crevicular fluid and mucosal transudate which infiltrate the saliva due to oral and systemic diseases. Nowadays many assays are available to analyse various salivary parameters, however, standardization of collection and storage methods is essential to obtain meaningful results. The relatively easy non-invasive nature of collection and the relationship of saliva with plasma levels make saliva an attractive diagnostic tool. Type of saliva; stimulated or unstimulated will have a significant effect on salivary biomarker composition and analysis and this needs to be taken in to consideration when using saliva for diagnostic/ screening purposes. Future developments in salivary diagnostics for screening, risk assessment and therapeutic management for a range of conditions are promising and this approach will hopefully allow individualized treatments before arise of major clinical symptoms

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