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SALIVA - A VERSATILE DIAGNOSTIC TOOL IN HEALTH AND DISEASE

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ABSTRACT

Saliva presents an increasing area of research with implications for basic and clinical purposes. Saliva is potential diagnostic fluid that offers advantage over serum and other biological fluids by an economic and noninvasive collection method for monitoring of systemic health and disease progression. The plethora of components in this fluid can act as biomarkers for diagnosis of various systemic and local diseases. In this review paper, we have emphasized the role of salivary biomarkers as a versatile diagnostic tool. Saliva use in assays will continue to expand thus providing a new instrument of investigation for physiologic as well as pathophysiologic states.

KEYWORDS: Saliva, biomarker for different disease.

INTRODUCTION

Human diseases have global impact. Diagnosing disease like cancer, cardiovascular, metabolic, and neurological diseases is becoming challenging, thus requires clinical evaluation with laboratory testing. Saliva as a diagnostic tool can be used as an effective modality for the early diagnosis, prognostication, and monitoring post therapy status. The main advantages of saliva as a clinical tool over serum and tissues are (i) noninvasive

collection of sample, (ii)smaller sample aliquots, (iii)good cooperation with patients, (iv)cost effectiveness, (v)easy storage and transportation, (vi)greater sensitivity and correlation with levels in blood.

Saliva is a mixture of the secretions of the major and minor salivary glands, mucosal transudations, gingival crevicular fluid, serum and blood derivatives from oral wounds, desquamated epithelial cells, expectorated bronchial and nasal secretions, bacteria and bacterial products, viruses and fungi, other cellular components, and food debris. It is a complex fluid containing hormones, proteins, enzymes, antibodies, antimicrobial constituents, and cytokines, which enter from the blood into the saliva by transcellular, passive intracellular diffusion and active transport, or paracellular routes by extracellular ultrafiltration within the salivary glands or through the gingival crevice.[1, 2, 3] Some of the technologies have unveiled large numbers of medically valuable salivary biomarkers for different disease conditions including cancer, autoimmune, viral, bacterial, cardiovascular, and metabolic diseases.

Salivary diagnostics can be used for the following diseases/conditions *Cardiovascular Diseases*- C-reactive protein (CRP), myoglobin (MYO), creatinine kinase myocardial band (CK-MB), cardiac troponins (cTn), and myeloperoxidase, are salivary markers of cardiovascular diseases which, when used in combination with an ECG, shows a positive correlation with myocardial infarct patients as compared to healthy controls. The salivary MYO levels are significantly higher within 48 h of onset of chest pain in AMI patients. Furthermore, salivary MYO levels are correlated positively with its serum concentrations.[4] Miller et al., found that the salivary concentrations of CRP, TNF-α, and MMP-9 were significantly higher in patients with AMI and the salivary concentrations correlated positively with the serum concentrations. In addition, salivary myeloperoxidase levels were also found to be elevated in AMI patients. Studies have revealed that salivary soluble ICAM-1 is significantly elevated in AMI patients whereas salivary soluble CD40 ligand is significantly lower in AMI patients.[5] Salivary lysozyme have been shown to be increased with hypertension, an early stage of cardiovascular disorders.[6,7]

*Autoimmune Diseases-(i) Sjogren's Syndrome (SS)*Sialochemistry offers great value in the diagnosis of autoimmune disorder SS, which is characterized by reduced secretion of the salivary glands and lacrimal glands and associated endocrine disturbance. An increase in the levels of immunoglobulins, inflammatory mediators, albumin, sodium, and chloride and a

decrease in the level of phosphate are indicative of SS. In another study salivary protein analysis demonstrated an increased level of lactoferrin, beta 2 microglobulin, lysozyme C, and cystatin C, while salivary amylase and carbonic anhydrase levels were decreased.[8,9] *(ii) Multiple Sclerosis*

Multiple sclerosis (MS) is an inflammatory disease characterized by loss of myelin and scarring caused due to destruction/failure of myelin producing cells by the immune system. Salivary diagnostics shows a reduction in IgA production.[10] *(iii) Sarcoidosis* Sarcoidosis is an inflammatory disease of the lymph nodes, lungs, liver, eyes, skin, or other tissues. Salivary diagnostics demonstrates a decrease in the secretion volume of saliva in addition to a reduction in the enzyme activity of alpha-amylase and kallikrein in most of these patients.[11]

Periodontal Diseases-Saliva monitors the level of oral bacteria. The increased numbers of Streptococcus mutans and lactobacilli in saliva have been associated with increased caries prevalence and root caries.[12] Periodontal diseases have been associated with increased levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Lower levels of uric acid and albumin in the saliva were associated with periodontitis and diabetes.[13] In patients with type 2 diabetes mellitus, the salivary expression of pIgR, Arp 3, CA VI, and IL-1Ra was downregulated, whereas PLS-2, LEI, and IGJ chain appeared to be upregulated.[14]

Metabolic bone disorder-Saliva can be used in mass screening for metabolic bone disorder. Significant correlations have been reported between age, body mass index, D-PYR, or OC concentration and calcaneus T scores suggesting saliva could be used as a fluid for assay of human biomarkers of bone turnover. Positive association was reported between alveolar bone loss and salivary concentrations of hepatocyte growth factor and interleukin-1 beta. The increased levels of alkaline phosphatase (ALP) activity in periodontitis have been correlated with the alveolar bone loss.[15, 16]

Drug Level Monitoring-Saliva is used for drug monitoring and detection of illicit drugs. Saliva is used to detect the presence of nicotine, cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepines, amphetamines, and ethanol. In drug level monitoring, only the unbound fraction of the drug in serum diffuses into the saliva and is detectable in the saliva. The drug appears in the saliva during the same duration as the serum and thus its mere presence is satisfactory for forensic purposes.[17, 18] The major metabolite of nicotine, cotinine, present in the saliva was found to be indicative of active and passive smoking.[19]

Rapid detection of illicit drug use can also be done through the direct analysis of methamphetamine, cocaine, and 3,4-methylenedioxymethamphetamine in saliva by a hydrophobic porous silicon array.[20] The γ-hydroxybutyric acid (GHB) in the blood and urine has posed problems for the forensic toxicologist however Saliva is of great importance for drug testing of GHB levels because of its merit of easy, noninvasive collection and stability of the drug.[21]

Forensics- The salivary samples can be easily obtained from glasses, cigarettes, food products, envelopes, and other sources. Blood group antigens are secreted into the saliva which can be used for the identification of crime suspects and for paternity law suits,[22] Identification of DNA in saliva by genetic profiling can be helpful in cases of sexual abuse and harassment. The foreign DNA tends to be present in the victim's saliva for as long as 60 minutes providing a valuable piece of forensic evidence.[23]

Genetic Disorders- Cystic fibrosis (CF) is caused due to a mutation in the CFTR gene. Saliva is modified in CF patients. The CFTR protein is expressed in the epithelial cells of the parotid gland causing parotid gland involvement. The level of activity of cathepsin-D in saliva of CF patients is significantly higher than in healthy controls. Salivary calcium concentration, magnesium concentration, and lactate dehydrogenase levels were increased in CF patients when compared with healthy controls.[24,25] *Ectodermal Dysplasia* is the X-linked hypohidrotic ectodermal dysplasia (HED) in which there is reduced whole saliva flow and the concentration of inorganic constituents.[26]

Renal Diseases-The various salivary markers associated with end stage renal disease included nitrite, pH, sodium, chloride, uric acid, cortisol, alpha-amylase, and lactoferrin.[27] Salivary phosphate has been widely used as a clinical biomarker for hyperphosphatemia. These levels have correlated well with serum creatinine and glomerular filtration rate.[28,29]

Malignancy-Early detection is the key to good prognosis in almost all types of cancer. Saliva has been used as a diagnostic medium for oral squamous cell carcinoma (OSCC), and salivary analytes such as proteins, mRNA, and DNA have been used in their diagnosis. Aberrant expressions of long noncoding RNA (lncRNA) are associated with lung, breast, and prostate carcinomas. In a study, saliva contained a detectable amount of lncRNA which could be potential markers of OSCC,[30] Salivary mRNA biomarkers (CCNI, EGFR, FGF19, FRS2, and GREB1) can aid in the noninvasive and economical diagnosis of lung cancer. The

salivary mRNA biomarkers for detection of ovarian cancer are AGPAT1, B2M, BASP2, IER3, and IL1B.[31,32] The p53 antibodies can be detected in the sera and saliva of patients diagnosed with OSCC. CA15-3, tumor marker is found in the saliva of women diagnosed with breast cancer. Elevated levels of c-erb-2 tumor marker are found in saliva of patients diagnosed with breast cancer when compared with patients with benign lesions and healthy controls.[33, 34, 35] c-erbB-2 soluble fragments and 15-3 cancer antigen in breast cancer research have been demonstrated in saliva.[36] The profiling of salivary RNA transcript with microarray analysis seems to be able to distinguish several genes exhibiting significantly different expression levels in saliva comparing

oral squamous cell carcinoma patients with controls.[37] Fibroblast growth factor 2 (FGF2) and fibroblast growth factor receptor 1 (FGFR1) concentrations in saliva are significantly elevated in patients with salivary gland tumors making it a potential biomarker in the early detection of salivary gland tumors.[38]

Salivary Prostate specific antigen PSA levels correlate with serum PSA levels in patients with PA. This can serve as a useful biomarker of PA.[39]

Salivary cortisol levels and Salivary lactate dehydrogenase levels were found to be significantly increased in the plasma and saliva of patients of OSCC.[40] Elevated levels of salivary nitrate and nitrite were found in patients with oral cancer.[41] Salivary adenosine deaminase (ADA) activity is significantly increased in squamous cell carcinoma of the tongue.[42]

Viral / Bacterial /Fungal Infections- The oral mucosal transudate (OMT) which is obtained by swabbing the buccal mucosa and tongue contains a mixture of sIgA, IgG, IgM, and a rich source of antibodies.[12] Measles virus-specific IgM detection in the saliva is a suitable noninvasive method for routine clinical use.[43] Diagnosis of HIV (human immunodeficiency virus) in which confirmatory test is a reactive antibody assay which is either a Western blot test via blood or saliva or a polymerase chain reaction via blood. These tests detect p24 antigens and antibodies against both HIV-1 and HIV-2.[44] Detection of Mycobacterium tuberculosis in the saliva is done by Polymerase chain reaction during the acute phase of the disease when the bacterial load is high.[12, 45] Helicobacter pylori (H. pylori) is Gram-negative, microaerophilic bacterium found in patients with chronic gastritis and gastric ulcers. H. pylori binds to salivary mucins MUC-5B and MUC 7 secreted by the mucous and

serous acinar cells of the seromucous salivary glands, respectively. Higher levels of salivary MUC-5B and MUC 7 could be used as an indicator for infection with H. pylori.[46, 47] The salivary fungal count analysis and the alterations in the salivary proteins, like immunoglobulins, Hsp70, calprotectin, histatins, mucins, basic proline rich proteins and peroxidases also have important diagnostic value in oral candidiasis.[48, 49]

Occupational and Environmental Medicine-Chronic stress is associated with increased levels of salivary cortisol and decreased level of salivary IgA and lysozyme. Saliva chromogranin (Cg) A and alpha-amylase are markers of acute stress.[50] Occupational toxins such as lead and cadmium can also be analyzed from the saliva.[51,52] There is an increase in the level of salivary amylase and a decrease in the secretory IgA levels in conditions of psychological stress,[8] Salivary testosterone levels have been correlated with the aggressive behavior and athletic activities.[53]

CONCLUSION

Saliva as a diagnostic tool is an upcoming area of research for basic and clinical application purposes. Saliva is a biological fluid that offers several opportunities in diagnosis, toxicology and in forensic science. Furthermore, many salivary proteins offer great potential in clinical and epidemiological research, in oral as well as in general health studies.

The greatest milestone in salivary diagnostics is to identify the disease biomarkers and to transfer it from the laboratory to the clinical practice. The improved efficiency of genomic and proteomic technologies, has made easier the use of salivary diagnostics in a clinical setting. Salivary metabolomics analyzes a large array of low molecular weight endogenous metabolites present in the saliva for the detection of diseases. Oral Fluidic NanoSensor Test (OFNASET) by the UCLA Collaborative Oral Fluid Diagnostic Research Center is used for the detection of multiple salivary protein and nucleic acid targets in disease conditions.

The growth of salivary diagnostics has been hindered because of lack of sensitive detection methods, lack of correlation between the biomolecules in the blood and saliva, and the circadian variations in saliva. However, unlike blood and other body fluids, salivary diagnostics offers an easy, inexpensive, painless, and stress free approach to disease detection

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