Sialochemistry – An Emerging Oral Diagnostic Tool
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Abstract: Saliva, a non-invasive bio-fluid secreted by the salivary glands not only lubricates the oral cavity but also has antibacterial, antifungal and antiviral properties and also regulates pH. As a diagnostic fluid, saliva offers distinctive advantages over serum because individuals with modest training can collect it non-invasively. This review examines the diagnostic application of saliva for hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use, and also for forensic evidence and others.

Key words : Saliva, Diagnosis, Diseases, Disorders, Alcohol, Caries

INTRODUCTION
Most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Saliva offers some distinctive advantages when used for diagnosis of disease. A large number of diagnostic analytes have been shown to be present in saliva, including steroid hormones and the HIV antibody. For the past two decades, oral health researchers have been developing salivary diagnostic tools to monitor oral diseases including periodontal diseases, as well as for caries risk assessment. These diagnostic advances range from genetic susceptibility analysis of interleukin-1 (IL-1) genetic alleles to the analysis of oral pathogens identified via lectin staining for caries risk assessment. The current development of diagnostic biomarkers (via proteomic and genomic approaches) in conjunction with technological developments in salivary diagnostics will lead to the development of robust diagnostic tools for dentists to use in making clinical decisions and predicting treatment outcomes.

COLLECTION OF SALIVA
Whole saliva is a product of secretion of 3 major glands (parotid, submandibular, sublingual) and many minor glands (labial, buccal, palatal). Unstimulated saliva is usually obtained as the patient spits out every 60 sec or by forward bended head, the patient allows saliva to drip off the lower lip into a cylinder. By collection of saliva in the tube the flow rate per unit time can be measured. When volume measurement is not required the saliva can be collected on cotton rolls, gauze or filter paper. For evaluating salivary gland function or when large volumes of saliva are required for analytic purposes, stimulated whole saliva is used. Method of collection is the same as for unstimulated saliva. The usual masticatory stimuli are paraffin wax or a washed rubber band. A standard gustatory stimulus is obtained by 2% citric acid applied directly to the tongue every 15 to 60 sec. Parotid saliva can be collected by aspiration from the duct opening with a micropipette. Parotid saliva is best collected with Lashley’s vacuum. Submandibular and sublingual saliva can be collected by cannulation of the duct with micropipette, but in practice this is both uncomfortable for the patients and technically difficult since the duct orifice is mobile and has a strong sphincter. Because of that, alginate and silicone impression material is used for retention of the collecting tube. As alternative and simple technique is to block off secretion from the parotid glands with absorbent swabs and collect mixed submandibular and sublingual saliva by pipette from the floor of the mouth. Saliva from labial and palatal glands can be collected by filter paper disc or disc of other synthetic materials.

Normal Salivary flow rate is 1-1.5 ml/min. When the rate increases and decreases it is called as sialorrhoea and xerostomia respectively. The flow rate also helps in diagnosis of various systemic diseases.

SIALOCHEMISTRY
The diagnostic use of saliva has attracted the attention of numerous investigators because of the noninvasive nature and relative simplicity of collection. Saliva collection also simplifies the diagnostic process in special populations in whom blood drawing is difficult, i.e. individuals with compromised venous access (e.g., injecting drug users), patients with hemophilia and children. The presence of six enzymes was established in parotid saliva: acid phosphatase, total esterases, cholinesterase, lipase, beta-glucuronidase, and lysozyme. Broth cultures used for this study with whole saliva indicated that all but sulfatase and beta-glucuronidase were produced by the oral flora.

Abnormal proteins are also produced under exceptional conditions, such as the development of tumors and nutritional deficiency. Low a-amylase concentrations are seen in cases of starvation and after destruction and degeneration of the acinar cells. Elevated amylase is seen in abnormal ductal water loss. Furthermore, acute inflammation of the glands produces a rise in plasma and urine amylase due to gross glandular leakage. This will be seen in mumps as well as in the presence of a salivary calculus. Salivary analysis can be valuable to discriminate and monitor swellings of major salivary glands such as chronic recurrent parotitis, where the blood-saliva barrier is violated in the inflammatory flare-ups and characterized by the leakage of
Serum components (albumin) and lactoferrin into saliva.11

Sialochemistry can be expected to reveal the differentiation between normal and abnormal function of the glands, information about gland dysfunction and its impact on the oral environment, clues to homeostatic fluctuations as a result of circulatory, innervatory, or hormonal adjustments. The development of microchips for salivary components offers great possibilities to use oral fluid for point-of-care testing. Another fertile area of application for salivary analysis is in laboratory medicine, where determining and monitoring levels of various hormones (cortisol, progesterone, estradiol, testosterone etc.) and drugs (diazepam, caffeine, lithium, theophylline, tobutamide, methotrexate, antibiotics, anticonvulsants, etc.) is becoming a conventional procedure. Saliva may be used for monitoring patient compliance with psychiatric medications. A significant correlation exists between the salivary and serum lithium levels in patients receiving lithium therapy. Saliva is also useful for the monitoring of anti-epileptic drugs. Salivary carbamazepine levels showed positive correlation with serum levels. In another study, salivary levels of Phenobarbital and phenytoin demonstrated excellent correlations with serum levels of these medications. Salivary theophylline concentration demonstrated correlation with serum concentration of theophylline. Saliva is increasingly being used as an investigational aid in the diagnosis of systemic diseases that affect the function of the salivary glands and the composition of the saliva, such as Sjorgen's syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus and diseases of the adrenal cortex. In the past 10 years researchers have demonstrated that saliva tests for anti-bodies to HIV represent a noninvasive alternative to quantification of antibodies in blood for monitoring the efficacy of antiretroviral therapies and disease progression to acquired immunodeficiency syndrome.15

Patients with heart diseases were divided into 2 groups: Warfarin user and Warfarin non-user, and six components of saliva: salivary total protein (TP), albumin(ALB), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK) were determined to assess intraoral pathologic conditions. As a result there were many cases showing a high ALB, CRP, AST and ALT level in Warfarin user and significant correlation found between clinical parameters and salivary components.16

Human saliva contains proteins that can be informative for disease detection and surveillance of oral health. Recent studies have shown that several species of oral streptococci, such as Streptococcus gordonii, bind to salivary alpha-amylase immobilized onto hydroxyapatite surfaces. Thus amylase, as a receptor for streptococcal adhesion to the tooth, contributes to the formation of dental plaque.5 p53 antibody is a tumor suppressor protein which is produced in cells exposed to various types of DNA-damaging stress, can also be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection and screening for this tumor. Higher concentrations of salivary defensin-1 were detected in patients with oral SCC in comparison to healthy controls.18

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. Detection of certain bacterial species in saliva can reflect their presence in dental plaque and periodontal pockets. The Oral Fluid Nano Sensor Test (OFNASET) technology platform combines cutting-edge technologies, such as self-assembled monolayers (SAM), bio nanotechnology, cyclic enzymatic amplification, and microfluidics, with several well established techniques including microinjection molding, hybridization-based detection, and molecular purification. The UCLA laboratory recently discovered that discriminatory and diagnostic human mRNAs are present in the saliva of healthy people and people with disease. The salivary transcriptome offers an additional valuable resource for disease diagnostics. The behavior of these salivary transcriptome biomarkers is consistent i.e. their levels are significantly higher in the saliva of patients with oral cancer than in the saliva of matched control subjects, however, before a salivary diagnostic test can replace a more conventional one, the diagnostic value of a new salivary test has to be compared with accepted diagnostic methods.20

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. Saliva can also be used to detect recent marijuana use by means of radioimmunoassay. Monitoring of salivary albumin can assist in the identification of stomatitis at a pre-clinical stage and enable the chemotherapy dosage to be adjusted or treatment for the stomatitis to be initiated at an early stage.21

CONCLUSION

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the old and in large scale screening and epidemiologic studies. The saliva has found use as a diagnostic aid in an increasing number of systemic diseases that can affect salivary gland function and composition. Therefore a correct diagnosis will always require a full clinical and laboratory investigation. However, sialochemistry is a useful means of chronologically, monitoring qualitative and quantitative changes.

REFERENCES

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