A Revolution in Biomedical Assessment: The Development of Salivary Diagnostics

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Abstract: Since the early 1900s, saliva has proven to be a noninvasive medium from which to measure a wide range of hormones, pharmaceuticals, and antibodies. It has also proven to be a convenient source of host and microbial DNA. As we enter the era of genomic medicine, increasing use of salivary diagnostics will help catalyze a shift from disease diagnosis to health surveillance. However, with the advances in this technology comes the additional obligation to ensure the privacy and rights of patients.

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Key words: sialochemistry, diagnostics, health surveillance

The accessibility of the mouth renders it a portal through which one can potentially monitor systemic and oral health. A comprehensive review of saliva as diagnostic fluid was published in 1993. We will use this brief review as a means of highlighting some areas of progress since that date and offering suggestions for future directions.

Overwhelmingly, investigators have employed some form of “whole saliva,” that is, not collected from one specific major or minor salivary gland. For the purpose of this review, “whole saliva” is defined as whole mouth fluids consisting of an admixture of the secretions of the major parotid, submandibular, and sublingual glands; the secretions of numerous minor salivary glands located in the palate, buccal, and labial mucosa; the crevicular fluid; and the fluids resulting from mucosal seepage.

The earliest “sialochemical” studies on oral fluids were conducted by Micheals and Kirk, each of whom examined saliva for specific components that would be diagnostic for various systemic conditions, including gout and rheumatism. Howe appreciated the potential value of collecting human secretion from a single (parotid) gland; he studied the secretion of several antimicrobials, including salicylates and benzoates.

Insight into a wide range of clinical situations may be gained from salivary analysis. A recent example makes use of salivary acetaminophen concentration to assess the gastric emptying rate of liquids. There are a number of current reports using saliva specimens for the monitoring of pharmaceuticals, including taxol, caffeine, and the protease inhibitor indinavir. However, the “summary statement” of sialochemistry in the twentieth century is perhaps best summed up in the title of a seminal review written by Irwin Mandel, “Salivary Diagnosis: Promises, Promises.” In this review, Mandel outlined several cardinal rules of salivary diagnosis:

1. If no one uses the test, it is not useful;
2. Changes in salivary composition in a disease state could provide insight into the pathogenesis of that disease; and
3. Just because clinicians do not elect to use a salivary test does not mean that it is not a valid diagnostic aid.

Rules 1 and 3 speak to the issue of the general nonusage among dentists and physicians of saliva-based tests. Colin Dawes has argued that “Saliva collection and analysis will not become accepted in North America until there is greater emphasis on oral diagnosis and assessment of disease susceptibility rather than on immediate treatment, and until saliva studies are incorporated into the routine diagnostic procedures employed in dental schools and subsequently by the dental profession.” It is not surprising, therefore, that the overwhelming majority of saliva-based tests are driven largely by exploiting the relative ease with which the specimen can be collected. Although the test is technically noninvasive, it must be emphasized that issues related to patient confidentiality must be considered. The facility to collect samples from very young or very old subjects does not provide the license to suspend a patient’s right to privacy.
Where Facile, Noninvasive Sampling Is Desired, Saliva Is the Medium of Choice

Detection and Measurement of Drugs

Many analytes, including drugs of abuse, can be measured in saliva and oral fluids. Particularly useful where a “yes/no” answer is required, oral fluid-based tests find wide usage in detection of recreational drugs, including alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, a variety of inhalants, lysergic acid diethylamide (LSD), marijuana, opioids, phencyclidine (PCP), and tobacco. Most recently, law enforcement agencies have employed saliva-based tests for roadside evaluation of alcohol levels, and it has been suggested that saliva-based alcohol tests be employed in hospital emergency departments as a rapid means of determining whether impaired consciousness is related to alcohol intoxication. Tobacco usage or exposure (via “passive” or “secondhand” smoke) is now routinely measured by quantitation of levels of salivary cotinine that are shown to have similar clearance and half-life values as plasma.

Detection and Measurement of Hormones

Numerous studies have validated methods to accurately assay various hormones from salivary samples. Several recent studies have underscored the ease of saliva sampling as a means to assess ovarian function. A recent prospective, longitudinal, masked multicenter trial of 956 women found that a single positive salivary estriol test predicted an increased risk of spontaneous preterm labor and delivery in both asymptomatic and symptomatic women. The circadian rhythm of salivary cortisol levels has been studied in relationship to sleep activity in preterm infants. The investigation demonstrated that the rhythm appeared at the same postnatal age as reported for term infants. Individuals with Cushing’s syndrome present with changes in their diurnal cortisol variation and complete or partial resistance to dexamethasone. Two recent papers demonstrate that nighttime salivary cortisol sampling is a facile approach to screening for Cushing’s syndrome in children. Abnormal circadian rhythms have also been observed in cancer patients. Altered diurnal salivary cortisol levels have recently been shown to be predictive (albeit not causal) of breast cancer survival.

Plasma levels of the vasoconstrictor peptide endothelin-1 are increased in patients with chronic heart failure. A recent study has demonstrated that salivary endothelin concentrations are also elevated in persons with chronic heart failure, and the levels can be used to assess disease severity.

Perhaps the most profound impact has been in the area of behavioral science. Saliva collection affords a means of biosampling with minimal perturbation of the natural setting and in many instances in a less culturally intrusive way than is achievable by vein puncture. Interesting examples of this type of inquiry are seen in a number of recent papers in which salivary cortisol levels have been employed as an indicator of stress among subjects across the life span. For example, saliva cortisol levels were highest in infants delivered by assisted (for example, forceps) means and lowest in those delivered by elective cesarean section. Salivary cortisol levels have been used as one measure to assess the effectiveness of simple behavioral interventions at immunization of infants two to twenty-four months of age. The ease of sampling allowed for sequential collection and hence the opportunity to examine cortisol levels as a function of time after immunization. The level and rate of salivary cortisol change following immunization was lower for the infants enrolled in the intervention group. These biochemical measures of stress correlated well with behavioral indicators of stress as well. Neuroendocrine profiles were obtained for subjects experiencing military survival training using saliva samples collected at baseline and at four subsequent “stress” points. Cortisol levels increased significantly during the captivity experience and peaked following interrogation. Testosterone levels were significantly reduced within twelve hours of captivity. Numerous other mind-body interrelationships have been explored using salivary hormone levels as one parameter including anxiety disorders and depression.
Detection and Measurement of Antibodies

Detection of specific salivary and/or oral fluid antibodies forms the basis of tests for microbial infections. Ease of sample collection has led to considerable interest and use of saliva-based tests for HIV, particularly in the field. For example, a recent report from Yangon Myanmar determined that HIV antibodies in saliva were stable for one month at ambient temperatures when collected with a commercially available device (Omni-Sal, Saliva Diagnostic Systems, Inc.). In Jamaica, where cultural norms lead to a high reluctance to have any form of blood test, a commercially available saliva test (OraScreen™ HIV rapid test, Beacon Diagnostics) was piloted. This test showed 100 percent specificity identifying 256/256 HIV antibody negative individuals and 100 percent sensitivity identifying 52/52 infected individuals as HIV antibody positive. A saliva-based test was used to identify HIV prevalence (2.2 percent) among 695 Toronto street youth, aged fourteen to twenty-five years.

Saliva-based tests are also finding use in diagnostic and epidemiology studies of herpes viruses, hepatitis B virus, Epstein-Barr virus, and ameba (Entamoeba histolytica) infection.

Tests Employing DNA Derived from Cells Found in Whole Saliva

Oral fluid also provides a convenient source of microbial or human DNA; this has proven useful for both biomarker profiling and forensic identification. For example, mitochondrial DNA mutations found associated with primary tumors of the human bladder, head and neck, or lung can be detected in saliva. A recent report suggests that head and neck squamous cell carcinoma may be detected using microsatellite analysis of DNA derived from exfoliated oral mucosal cells sampled from saliva. A recent study confirmed that DNA methylation of the promoter of an orally expressed oncogene in oral cancer cells can be used as a sensitive and specific diagnostic test for oral cancer.

Analysis of DNA found in saliva left on human skin or on other evidentiary material such as postage stamps or envelope flaps has proven useful to forensic scientists in the identification of individuals.

For Oral Health Researchers, the “Medium” Is Also the Message

Although considerable progress has been made in using saliva as a means of assessing systemic conditions, a major remaining challenge in oral biology is to learn how best to use sialochemistry to assess the two most highly prevalent oral diseases: caries and periodontal disease. As pointed out almost a decade ago, we still do not know what the normal variation is for levels of salivary proteins. Most studies have been conducted on relatively small numbers of subjects and, given the marked variation in expression levels observed, it is clear that very large numbers of persons will need to be assayed for meaningful results to be obtained. The costs involved for studies involving large numbers of subjects are best performed in partnership with academia, industry, and public agencies. The potential benefit of a specific and sensitive diagnostic test to reveal those most at risk could have a profound impact on the allocations of health resources to those most at need.

Saliva Testing in the Genomic Era of Medicine

As we enter the era of genomic medicine, salivary diagnostics will play an increasingly important role in the early detection of disease, the monitoring of disease progression, and the evaluation of patient behavior including treatment compliance and lifestyle choice. Advances in bioengineering will replace the “outer-body” experience of in vitro testing with real-time, in vivo monitoring, using “smart” buccal patches, floss, or toothbrushes. This will facilitate the shift from a health care system concerned largely with disease diagnosis to health surveillance. With the advance in this technology comes the additional obligation to ensure the privacy and rights of patients.

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