Salivary Markers of Systemic Disease: Noninvasive Diagnosis of Disease and Monitoring of General Health

(Marqueurs salivaires des maladies systémiques : méthode non invasive de diagnostic et de suivi de l'état général de santé)

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Sommaire

Les cliniciens qui s'intéressent au lien entre la santé buccodentaire et l'état général de santé ont de plus en plus recours à des analyses salivaires pour diagnostiquer des maladies systémiques et surveiller l'état général de santé. Cet intérêt vient de la capacité des nouveaux outils de diagnostic, comme les dosages immunoenzymatiques sensibles et autres technologies, de déceler un éventail de constituants de la salive qui sont des biomarqueurs des changements de l'état de santé. Le caractère non invasif de ces analyses salivaires les rend d'autant plus intéressantes comme solution de rechange efficace aux analyses de sang et d'urine; de plus, il existe des trousses d'analyse à domicile que les gens peuvent utiliser pour surveiller eux-mêmes leur état de santé. Nous examinons dans cet article ce que la salive peut nous révéler sur l'état général de santé, en nous appuyant sur des exemples tirés de recherches récentes sur des biomarqueurs salivaires de maladies systémiques et en décrivant l'usage actuel — ainsi que les applications cliniques et expérimentales potentielles — des diagnostics basés sur les liquides buccaux.

Mots clés MeSH : *diagnostic techniques and procedures; saliva/chemistry; salivary proteins/analysis*

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Saliva is the product of multiple salivary glands lying beneath the oral mucosa. Each day, the human salivary glands produce almost 600 mL of serous and mucinous saliva[1] containing minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immunoglobulins (e.g., secretory immunoglobulin A [sIgA]), mucins and other glycoproteins.[1-5] Once saliva passes through the ducts and enters the oral cavity, it mixes with blood cells, microorganisms (viruses, bacteria and yeast) and microbial products, oral epithelial cells and cell products, food debris and upper-airway secretions. Although saliva consists mostly of water, it plays a key physiologic role in the lubrication and repair of the oral mucosa, the formation and swallowing of food boluses, the digestion of starch, the facilitation of food tasting and the control of oropharyngeal microbial populations. Saliva also aids in the formation of plaque and, through its supersaturation with respect to tooth mineral, in the process by which dental enamel can be remineralized.[6] At the same time, it possesses antimicrobial components and buffering agents that act to protect and maintain oral tissues. Proteins that are found in saliva, such as lactoferrin, lysozyme, peroxidase, defensins and histatins, can destroy or inhibit the growth of microorganisms in the oral cavity; a case in point would be the histatins, which have fungicidal properties.[7]

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.[8,9] These "salivary biomarkers" are being explored as a means of monitoring general health and in the early diagnosis of disease. Disorders and diseases in which saliva may aid in diagnosis include but are by no means limited to human immunodeficiency virus (HIV) seropositivity, cancer and preterm labour. Tests using saliva as a diagnostic tool have made substantial inroads into an array of clinical and research areas, such as virology, immunology, microbiology, endocrinology, epidemiology and forensics. In the past, serum has been the fluid most often used in disease diagnosis; however, saliva has many advantages over both serum and urine. For example, salivary assays for antibodies (to viruses and bacteria), unconjugated steroid hormones (e.g., estrogen, testosterone and progesterone), environmental toxins (e.g., cadmium, lead and mercury), tobacco (cotinine) and certain drugs (e.g., ethanol, theophylline and lithium) are sufficiently sensitive to accurately reflect the blood concentrations of these substances (for a review and summary tables, see Slavkin,[9] Mandel[10] and the proceedings of a recent conference on the subject[11]).

In the clinic or the laboratory, saliva is relatively easy to collect in sufficient quantities for analysis, and the costs of storage and shipping tend to be lower than those for serum and urine. Of course, a variety of factors may influence the rate of salivary flow and its physiologic characteristics, including circadian rhythms and activities such as exercise, and these factors should be taken into account when saliva is used as a diagnostic fluid.[12] For health care professionals and scientists, saliva tests are safer than blood tests, which are more likely to result in exposure to HIV or hepatitis. For the patient, the noninvasive collection techniques for saliva can dramatically reduce anxiety and discomfort, thereby simplifying collection of serial samples for monitoring general health and disease states over time. Interest in noninvasive home testing of saliva has led to a proliferation of Web sites offering saliva-testing kits; however, many of these kits have yet to obtain the approval of the Food and Drug Administration (FDA) or Health Canada.

In recognition of the importance of saliva as a diagnostic fluid, the New York Academy of Sciences sponsored a major conference on the subject in 1992.[11] The conference participants recommended the development of more sensitive and specific assays to measure and understand changes in saliva in relation to drug therapy and abuse, endocrine function, systemic and oral disease, genetic defects, nutritional status and age-specific changes. The conference raised awareness of the potential of saliva-based diagnostics, and continuing research has led to the development of more sensitive salivary assays, which have advanced our understanding of the association between oral health and general health.

Salivary Markers of Generalized Disease and Their Diagnostic Role

For decades, dental health professionals have used saliva to help assess the risk of caries by measuring its buffering capacity and bacterial content. Now, 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 Sjögren's syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus and diseases of the adrenal cortex. The introduction of polymerase chain reaction methods has led to the use of oral fluids as a source of microbial DNA for detecting viruses (e.g., the herpes virus associated with Kaposi's sarcoma[13]) and bacteria (e.g., *Helicobacter pylori*, which is associated with gastritis, peptic ulcers and possibly stomach cancer[14]). In addition, the onset and severity of infectious diseases can be determined by monitoring the presence of antibodies to the microorganisms found in saliva and the oral cavity. For example, in the past 10 years researchers have demonstrated that saliva tests for antibodies to HIV[15] represent a noninvasive alternative to quantification of antibodies in blood for monitoring the efficacy of antiretroviral therapies and disease progression to acquired immunodeficiency syndrome.[16]

Until recently, oral transmission of HIV through the saliva of infected individuals during dental treatment or as the result of biting or coughing has been considered less likely than vaginal or rectal transmission,[17] but concerns about this mode of transmission have been growing. Monitoring HIV loads through saliva tests, as an adjunct to blood tests, helps in identifying high levels of HIV in the oral cavity that might place the patient at risk of transmitting the virus orally. HIV-specific antibodies, nonspecific antimicrobial salivary defence factors and the recently discovered secretory leukocyte protease inhibitor have been found to inhibit HIV from invading cells,[18,19] and these components may explain how saliva can protect against oral contagion with HIV. However, the oral transmission of HIV through infected seminal fluid or milk deposited directly into the oral cavity may result from the dilution of the hypotonic saliva (which has low salt content) with the infected semen or colostrum, each of which has a higher salt content. In vitro, infected white blood cells in milk or serum usually swell and burst when exposed to fluids of lower salinity (such as saliva); however, when these fluids are introduced directly into the mouth, the protective salivary inhibition of HIV production by infected leukocytes is overcome.[17,20] Thus, the levels of protein and salt in saliva serve as important biomarkers of the body's ability to fight off infection. By monitoring these levels, clinicians and, with the advent of home testing, patients themselves can take the appropriate steps in combatting this type of viral infection.

Salivary proteins can also maintain an ecological balance among the diverse bacteria that affect oral and general health. Recent studies have shown that several species of oral streptococci, such as *Streptococcus gordonii*, bind to salivary alpha-amylase immobilized onto hydroxyapatite surfaces.[21] Thus amylase, as a receptor for streptococcal adhesion to the tooth, contributes to the formation of dental plaque. Typical respiratory pathogens, such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae* and *Haemophilus influenzae*, colonize the dental plaque of intensive care patients and residents of nursing homes.[22,23] Once established in the mouth, these pathogens can be aspirated into the lung and cause a nosocomial infection. The interaction among enzymes in the saliva that promote the adhesion and colonization of mucosal surfaces by respiratory pathogens may explain the potential role of oral bacteria in the pathogenesis of respiratory infection.[24-28] The example of amylase illustrates the important association between oral and systemic health, and points to the significant effect of surrogate markers of high bacterial levels in plaque and saliva that may lead to more severe forms of disease.

Yet another recent example of the role of saliva in the diagnosis of systemic health is evident in breast cancer research, where salivary testing for markers of the disease are being studied for potential use in conjunction with mammography. Saliva assays may soon be marketed for the protein c-erbB-2, which is a prognostic breast cancer marker assayed in tissue biopsies of women diagnosed with malignant tumours. The soluble fragments of the c-erbB-2 oncogene and the cancer antigen 15-3 were significantly higher in the saliva and serum of women who had cancer than in the saliva and serum of healthy controls and patients with benign tumours.[29] Pilot studies have indicated that the saliva test for this oncogene is both sensitive and reliable and is potentially useful in initial detection of and follow-up screening for breast cancer.[30]

In endocrinology, the ease of collecting saliva is simplifying serial measurements of hormone levels and their diurnal variation. In the past, salivary testing was less reliable than blood tests because of problems in precisely measuring levels of hormones in saliva. The presence of a small amount of blood in a sample (e.g., as a result of flossing or chewing food or gum) could compromise the results. However, the recent development of more sensitive radioimmunoassays has helped to minimize this problem. For example, salivary free-cortisol assays are now being perfected in the research setting and should soon be available to clinicians. It has been shown that salivary cortisol is a valid indicator of cortisol concentration in the serum and is not dependent on salivary flow rate.[31] Increased levels of circulating and salivary cortisol (the stress hormone) result from the activation of the hypothalamic–pituitary–adrenal axis brought on by psychological stress and extensive physical activity. Similarly, saliva is being used to detect a specific estrogenic hormone, estradiol, which has been found to predict preterm labour.[32] The FDA-approved test for estradiol can be used at home by women at risk for premature, low-birth-weight babies. Here again, salivary testing is being used to measure indicators of systemic conditions that help in explaining a particular hormonal response.

Expectorated whole saliva might one day replace blood as a suitable monitoring medium for nutritional deficiencies in elderly people. Older adults who suffer from malnutrition also exhibit signs of impaired immune response. Reduced resistance of the oral tissues to disease often leads to increased colonization by oral pathogens and to severe, sustained oral infections. Indeed, clinical signs of malnutrition and a compromised immune system frequently appear first in the oral cavity.[33] Dimeric sIgA, the predominant immunoglobulin in saliva, acts as the first line of defence in protecting against microbial invasion, largely by its ability to inhibit bacterial adherence to epithelial cells.[5,34,35] Studies of age-related changes in the composition of salivary secretions suggest that there are subtle changes in the protective capacity of salivary IgA antibodies, which make elderly people more susceptible to oral bacterial and fungal infections, such as root caries and candidiasis.[36-48] Moreover, significant age-related reductions in the concentration of mucins from the submandibular gland[49,50] could result in reduced lubrication and contribute to the sensation of mouth dryness that is commonly reported among older adults who are taking a number of medications.[51,52]

My research group has completed a pilot study to determine correlates of salivary sIgA in a sample of older adults living in a multilevel geriatric care centre. The goal of the study was to assess whether, after controlling for confounders such as functional dependence, number of prescription drugs per day and chewing ability, salivary factors (total sIgA, sIgA1, sIgA2, total protein and cortisol) could be significant markers for nutritional status.[53] We found that lower concentrations of protein-adjusted sIgA and higher concentrations of cortisol in stimulated whole saliva are associated with risk of malnutrition. In light of the growing number of older adults, analysis of saliva may offer a cost-effective approach for assessing declines in general health in geriatric populations.

Conclusions

With advances in microbiology, immunology and biochemistry, salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "mirror of the body's health," and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public. ❖

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