Systemic Assessments Utilizing Saliva: Part 1 General Considerations and Current Assessments

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In this 2-part review series, the current uses of saliva as a diagnostic fluid are reviewed, first with a focus on known measurements of systemic conditions. In Part 2, the role of saliva to measure bone turnover with a special emphasis on osteoporosis will be discussed. *Int J Prosthodont 2006;19:43–52.*

Whole saliva is a mixed fluid that is derived predominantly from 3 pairs of major salivary glands: the parotid, the submandibular, and the sublingual glands. Approximately 90% of total salivary volume results from the activity of these 3 pairs of glands, with the bulk of the remainder from minor salivary glands located at various oral mucosal sites. Whole saliva also contains gingival crevicular fluid (GCF), mucosal transudate; expectorated bronchial and nasal secretions; serum and blood derivatives from oral wounds, bacteria, and bacterial products; viruses and fungi; desquamated epithelial cells; other cellular components; and food debris. The sublingual glands contribute only 1% to 2% of unstimulated volume of whole saliva, and secretions are also mucin-rich and somewhat viscous. Saliva is approximately 99% water and 1% proteins and salts. Typically, normal daily salivary flow ranges from 0.5 to 1.5 L, with an average volume of 1 mL at any given time, meaning that a reasonable volume can be collected fairly easily.

The principal functions of saliva are lubrication to protect the oral mucosa, lubrication to optimize mastication, clearance of food and bacteria, cleansing of the oral cavity, solubilization of food to facilitate digestion, promote optimal swallowing movements, and dilution of detritus. Other functions include facilitating speech, neutralizing unwanted effects of acid on oral tissues by buffering actions, maintenance of supersaturated calcium phosphate concentrations, participation in enamel pellicle formation, antimicrobial and protective actions, and digestion. As such, saliva is involved in a wide range of crucial biologic processes and, not surprisingly, partial or complete xerostomia has significant adverse effects.

The source of certain salivary proteins is likely to be circulating serum. As a result, saliva has been used as a diagnostic biofluid specifically to measure host responses to a variety of physiologic events. In this 2-part review series, the current uses of saliva as a diagnostic fluid will be reviewed first, with a focus on known measurements of systemic conditions. In Part 2, the role of saliva to measure bone turnover with a special emphasis on osteoporosis will be discussed.

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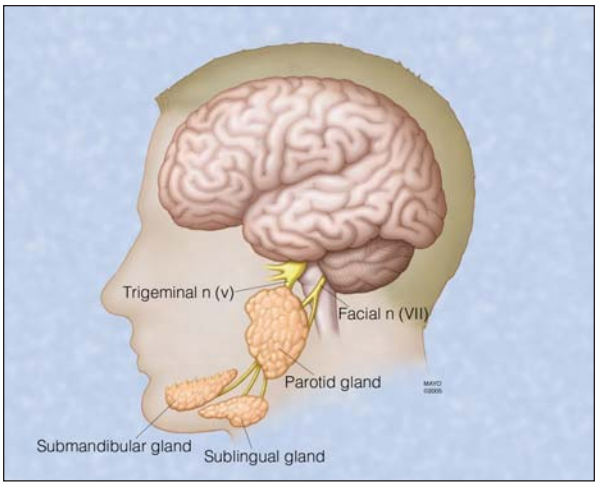

Fig 1 Typical locations and innervation pathways of salivary glands.

Neurophysiologic Control of Salivation

Parasympathetic Control

Parasympathetic innervation to the salivary glands originates in the salivatory nuclei of the brain stem (Fig 1).[1] Parasympathetic signals transmitted to the glands can be regulated by local and central input to the salivatory nuclei. Gustatory, olfactory, and mechanical stimuli transmitted to the medullary nuclei stimulate salivary gland secretion.[2,3] Central impulses triggered by the sight and smell of food also excite the salivatory nuclei and can induce salivation before food is ingested.[4]

Preganglionic parasympathetic fibers travel in cranial nerve (CN) VII to the submandibular ganglia, from which postganglionic fibers reach the sublingual and submandibular glands. Preganglionic parasympathetic fibers also travel in CN IX to the otic ganglia, from which postganglionic fibers reach the parotid glands. In addition, some parasympathetic fibers reach their final destination via the buccal branch of CN V to the parotid glands or via the lingual branches of CN V to the sublingual and submandibular glands.[1]

Sympathetic Control

Postganglionic sympathetic fibers from the superior cervical ganglia travel along blood vessels to the salivary glands.[4] The sympathetic nervous system is the primary stimulator of the myoepithelial cells that are closely associated with cells of the acini and proximal (intercalated) ducts. Myoepithelial cells are stellate shaped and have structural features of both epithelial and smooth muscle cells. The function of myoepithelial cells is to support the acinar structures and decrease the flow resistance of the intercalated ducts during stimulated secretion. The net effect of myoepithelial cell activation is to facilitate secretory flow in the proximal regions of the gland, minimizing the extravasation of secretory proteins that might otherwise occur during an acute increase in secretory flow.[1]

Mineralocorticoids also play a regulatory role in salivary secretion. The adrenal hormone aldosterone produces saliva that contains relatively less ionic sodium ions (Na^+) and more ionic potassium ions (K^+). The mineralocorticoid effect represents the only well-established example of a humoral agent in regulating salivary control.[1]

Neurotransmitter Control of Primary Saliva Formation

When a nerve to the salivary gland is stimulated, the transduction of this signal to increase the formation of saliva is first brought about by the release of a neurotransmitter. These substances are either sympathetic (noradrenaline) or parasympathetic (acetylcholine, substance P, and vasointestinal polypeptide).[4]

When the neurotransmitter arrives at a secretory cell membrane, it binds to, and activates a receptor on, the external surface of the membrane. These receptors can be characterized as stimulatory or inhibitory. Activation of the basolateral membrane receptor activates an intermediate guanine nucleotide-dependent membrane protein known as a "G protein,"[5] which in turn activates a regulatory enzyme on the inner cytoplasmic surface of the cell. The regulatory enzyme may be either phospholipase C (PLC) or adenyl cyclase.

PLC Pathway

PLC is activated as a result of acetylcholine binding to muscarinic receptors, substance P binding to peptidergic receptors, or noradrenaline binding to adrenergic receptors on the acinar cell membrane.[4] It controls the intracellular pathway that leads to the secretion of water and electrolytes.

Stimulation of PLC leads to the hydrolysis of a membrane phospholipid (phosphatidyl inositol 4,5-bisphosphate) to form 2 second messengers: diacylglycerol (DAG) and inositol triphosphate (IP^3).[5] DAG stimulates the release of calcium (Ca^{2+}) ions from the endoplasmic reticulum. This increased cytoplasmic Ca^{2+} ion concentration causes the opening of Ca^{2+}-activated K^+ channels in the basolateral membrane and chlorine (Cl^-) ion channels in the apical membrane.[6] This allows potassium chloride (KCl) to flow out of the cell, leading to an accumulation of Cl^- ions in the acinar lumen.[7,8] Na^+

is then thought to follow Cl^- by leaking from the interstitium through the tight junctions between the cells to preserve electroneutrality, and the resulting osmotic gradient for sodium chloride (NaCl) causes a transepithelial movement of water from interstitium to lumen.[7]

Adenyl Cyclase Pathway

Adenyl cyclase (AC) is activated when noradrenaline binds to β-adrenergic acinar receptors, or vasoactive intestinal peptide (VIP) binds to peptidergic receptors. Activation leads to exocytosis of secretory proteins.[5]

Adenyl cyclase activation results in intracellular formation of 3,5-cyclic adenosine monophosphate (AMP) from adenosine triphosphase. Cyclic AMP (cAMP) activates a second enzyme, cAMP-dependent protein kinase (cA-PK) or protein kinase A (PKA). The proteins targeted by activated PKA involved in exocytic secretion are unknown; however, PKA activation is essential for cAMP-dependent exocytotic secretion.[9,10] Diacylglycerol (from the PLC pathway) also promotes exocytosis.[5]

Processing of Primary Salivary Secretion by the Salivary Duct Cells

Thaysen et al[11] first described salivary fluid secretion as occurring in a 2-stage process. The first stage involves formation of a near-isotonic plasmalike[12] primary secretion in the acinar lumen, while the second stage results in production of a final hypotonic fluid that enters the mouth.

Salivary duct cells absorb Na^+ and Cl^- and, to a lesser extent, secrete K^+ and bicarbonate (HCO_3). Reabsorption of Na^+ by salivary duct cells is a 2-step process. First, Na^+ enters the cell from the lumen through Na^+ channels that are present on the apical membrane.[13] Second, the basolateral Na-K pump extrudes this Na^+ across the basolateral membrane,[14] thus providing a transcellular pathway for the movement of Na^+ from lumen to plasma.

Reabsorption of Cl^- is also a 2-step process. Entry of Cl^- across the apical membrane occurs by means of a Cl^-HCO_3 exchanger. Duct cells also have basolateral Cl- channels that provide an exit pathway for Cl^-, thus completing the movement from duct lumen to blood.[1] The basolateral step in K^+ secretion is the uptake of K^+ via the Na-K pump.[15] The apical step has been postulated to result from either a K-H exchange at the apical membrane, or a coupled exchange of K^+ secretion with Na+ re-absorption.[16]

The final composition of saliva secreted to the oral cavity becomes hypotonic with concentrations of Na^+ and Cl^- much below that of primary saliva.[17] Salivary concentrations of total protein, Na^+, Ca^{2+}, Cl^-, and HCO_3 are directly proportional to the flow rate of saliva.[18]

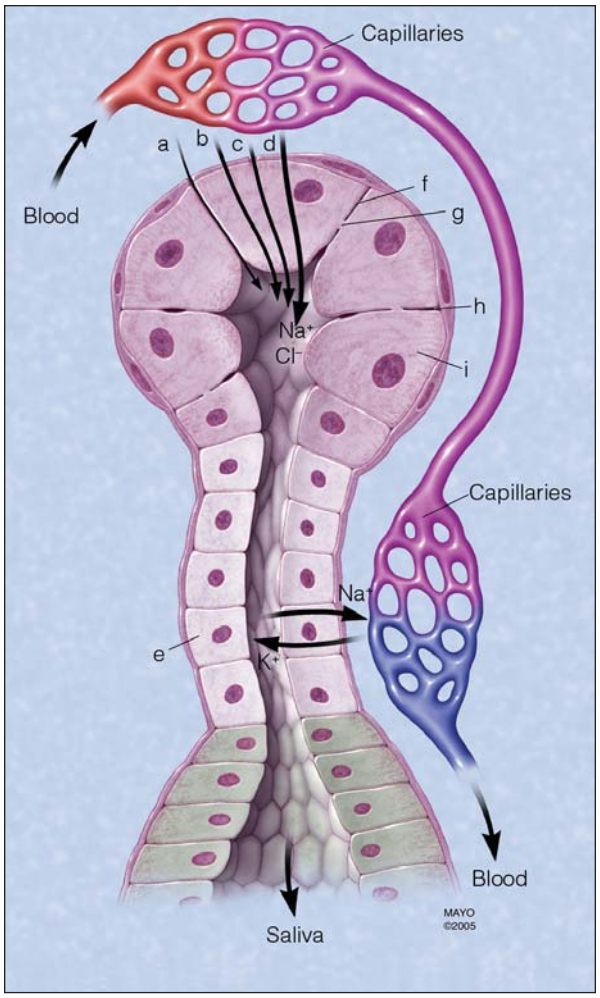

Fig 2 Mechanisms of transport of proteins and ions from serum into salivary gland ducts. A = ultrafiltration, b = active transport or passive diffusion across the cell membrane, c = simple filtration through cell membrane pores, d = transepithelial movement of water along NaCl gradient via channel proteins, e = creation of hypotonic salivary solution via ductal Na+ reabsorption, f = acinar cell membrane, g = cell membrane pore, h = intercellular space, i = acinar cell. (*Adapted with permission from Haeckel and Hanecke*.[23])

Sources of Sero-Salivary Constituents

Whole saliva is comprised of a number of constituents whose source is circulating serum (sero-salivary constituents).[19] The primary method of transfer from serum to saliva for many of these substances is passive diffusion through the membrane of the acinus cells of the salivary glands.[20–22]

Five barriers must be passed for a substance to travel from the vascular lumen to the ductal system where whole saliva is refined[23] (Fig 2):

1. The capillary wall
2. The interstitial space
3. The basal cell membrane of the acinus cell
4. The plasma of the acinus cell
5. The luminal cell membrane

There are at least 5 known factors that affect the diffusion of substances into saliva:

1. Molecular mass: Molecular mass appears to play a minor role in regulating diffusion. The diffusion coefficient is inversely proportional to the molecular radius.[22]
2. Lipid solubility: Lipophilic substances diffuse more easily than lipophobic substances.[24]
3. Degree of ionization: Nonionized or weakly basic substances diffuse more readily than acidic substances.[20]
4. Salivary pH value: Basic substances are concentrated in saliva under resting conditions when the salivary pH is below that in blood.[20,22]
5. Protein binding: Fractions of substances bound to protein are less able to pass across the cell membrane.[22]

Sero-salivary constituents also employ filtration through water-filled pores, active transport, or facilitated diffusion[22] as means to enter into whole saliva.

Current Systemic Diagnostic Applications of Saliva

Steroid Hormones

Passive diffusion through the salivary gland epithelium is the primary means of entry for steroid hormones into saliva.[25] The process of diffusion into the primary secretory fluid within the acinar-intercalated duct complex is driven by the free concentration of the hormone in the plasma. As the blood passes through the saliva gland during saliva formation, the free (unbound) or weakly bound (low-affinity binding protein) steroid hormone disassociates and passes through various membrane structures. Currently, the following steroids can be accurately assessed in saliva: cortisol,[26] dehydroepiandrosterone (DHEA),[27] testosterone,[28] estradiol,[29] estriol,[30] and progesterone.[31]

Cortisol. Salivary cortisol levels demonstrate excellent correlation with free serum cortisol levels,[32] which do not appear to be affected by changes in concentrations of serum-binding proteins. The actual salivary cortisol levels are lower than the serum-free cortisol levels. This is possibly a result of enzymatic degradation in the salivary epithelial cells during transcellular diffusion.[24]

Salivary cortisol levels have demonstrated usefulness in identifying patients with Cushing syndrome (CS). Diagnostic strategies applied to adult or pediatric patients with suspected hypercortisolism are usually based on measurement of urinary free cortisol, a low-dose or overnight dexamethasone (DEX) suppression test, and an evaluation of the diurnal variation of plasma cortisol.[33] Castro et al[33] collected salivary samples in adult and pediatric CS patients and age-matched controls at 11:00 pm and after an overnight DEX suppression test. In both instances, they demonstrated 100% sensitivity and specificity when the results of the 2 tests were combined.[33] Several studies have evaluated potential differences in diurnal cortisol excretion patterns in individuals experiencing chronic stress.[34–36] These studies demonstrate significant increases in cortisol levels upon waking within this population. In individuals experiencing acute episodes of stress, a positive correlation has been demonstrated between the stressor and elevated levels of salivary cortisol.[37,38] Levels of salivary cortisol have been studied to determine the adrenocortical response to exercise. It has been demonstrated that salivary and serum levels of cortisol are similar during exertion.[39,40]

DHEA and testosterone. Testosterone and DHEA have also been identified in saliva, with salivary concentrations demonstrated to be 1.5% to 7.5% of the serum concentrations of these hormones.[41] Salivary testosterone has been proposed as a means to assess testicular function.[42] Studies among male and female prison inmates have demonstrated relationships between salivary testosterone, violent crime, and misbehavior.[43] Salivary testosterone has been demonstrated to correlate with occupation.[39] For example, in a series of studies, Dabbs[43] determined that professional athletes, actors, and trial lawyers had higher levels of salivary testosterone than did ministers and non-trial lawyers.

Estradiol, estriol, and progesterone. Estradiol, in its unbound form, can readily diffuse into saliva. It has been detected in saliva at concentrations that are 1% to 2% of total serum estradiol concentrations.[19] These concentrations are similar to the serum concentrations of unbound estradiol.[44] Salivary estradiol levels have been shown to follow the same trends as serum estradiol levels during a menstrual cycle.[19]

Salivary estriol levels correlate well with levels of unbound serum estriol in pregnant women; consequently, salivary estriol levels have been suggested as a means for the assessment of feto-placental function.[44] Decreased salivary estriol has demonstrated usefulness as a predictor of low infant birthweight.[45]

Salivary progesterone levels also demonstrate good correlation with serum levels during the menstrual cycle

Table 1 Drugs Currently Monitored in Saliva

Therapeutic drugs	
Barbituates	Methadone
Benzodiazepines	Methotrexate
Caffeine	Metoprolol
Carbamazepine	Paracetamol
Cisplatin	Phenytoin
Cyclosporin	Primidone
Diazepam	Procainamide
Digoxin	Quinine
Lithium	Sulfanilamide

and reflect unbound serum progesterone levels.[46] Analysis of salivary progesterone levels has proven useful in diagnosing and treating infertility by providing an index of ovarian function,[47] and analysis of salivary progesterone levels can be employed as a means to diagnose luteal-phase insufficiencies.[48]

Systemic Disease

Alzheimer disease. Acetylcholinesterase (AChE) is the enzyme responsible for the catalytic reaction that converts acetylcholine (ACh) into its constituent components, acetic acid and choline. It has been postulated that decreases in ACh levels reflect the degeneration of cholinergic neurons in the early stages of Alzheimer disease (AD). Current chemotherapeutic management of AD involves administration of agents, such as donepezil hydrochloride (Aricept, Eisai), that inhibit the activity of AChE, thereby increasing the activity of released ACh. In assessing central cholinergic function, most studies have examined cerebrospinal fluid obtained by lumbar puncture. Sayer et al[49] analyzed levels of salivary AChE in 22 subjects (mean age 75) with mild dementia who responded to AChE inhibitor therapy, 14 subjects (mean age 75) who did not respond to AChE inhibitor therapy, and 11 age- and sex-matched controls. The findings led the authors to conclude that salivary AChE activity may prove to be a useful marker of AD-ssociated changes in central cholinergic activity and the responsiveness of patients to treatment with AChE inhibitors.

Drugs of Abuse/Chronic Dependence

Saliva has proven clinically useful for the monitoring of systemic levels of drugs (Table 1). Detectability of a drug in saliva is influenced by factors such as its molecular size, lipid solubility, and degree of ionization and protein binding, as well as the salivary pH.[21]

Passive diffusion is the primary means through which drugs enter saliva. Drug molecules diffuse more readily into saliva when they have a small molecular volume and are lipophilic, nonionized within the pH range of saliva, and non-protein bound.[23] Levels of alcohol, opioids, and nicotine (cotinine) are commonly assessed via salivary monitoring. Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine, and lysergic acid diethyalmide.[50] Delta 9-tetrahydrocannabinol (Δ9-THC), a major psychoactive component of marijuana, can be detected in saliva.[51] Detection times for Δ9-THC in saliva can range from 2 to 10 hours.[50]

Alcohol. Ethanol is unionized in serum, is not protein-bound, has a low molecular weight, and is lipid soluble. As a result, it readily diffuses into saliva, and the saliva-to-serum ratio is typically about 1.[23] When a salivary sample is obtained at least 20 minutes after ingestion, salivary ethanol concentration may be used as an index of the blood ethanol concentration. The 20-minute waiting period allows for absorption and distribution of alcohol, preventing falsely elevated readings resulting from oral consumption.[19]

Emergency room settings are a unique environment where determination of blood alcohol concentration (BAC) via salivary testing proves valuable. Patients frequently present in a combative or incoherent state, making them less compliant to administration of an alcohol breath meter. Degutis et al[52] administered alcohol saliva tests in 100 patients presenting to the emergency room. The collection device (QED A3[50]; STC Technologies) was placed into the mouth adjacent to the buccal mucosa and left in place for 2 minutes. The results were then analyzed according to the manufacturer's instructions. Serum samples from these same patients were analyzed for BAC simultaneously. Based on comparisons between the 2 methods in this patient group, these researchers concluded that the QED A350 saliva test could be used to measure BAC with an upper limit of 350 mg/dL. A similar study design that used control groups (patients who were asked to abstain from alcohol consumption for 24 hours prior to assessment) produced comparable results.[53]

Opioids. Codeine can be detected in saliva following oral and intramuscular administration. In one study, the mean saliva-to-plasma ratio for codeine for 3 individuals following an oral dose of 30 mg of codeine was 3.3.[50] Cone reported peak concentrations of codeine in saliva of 307.6 ng/mL and 183.9 ng/mL occurring at 30 to 45 minutes after doses of 120 and 60 mg of codeine, respectively.[54] Plasma concentrations in the same subjects peaked at 15 to 30 minutes and were below assay sensitivity by 36 hours.

Jehanli et al[55] administered controlled doses of codeine (16 mg) in 5 patients. The authors indicated that codeine was chosen as a model opiate because it possessed similar pharmacokinetic parameters to heroin, 6-monoacetyl morphine, and morphine, while at the same time had relatively safe and benign pharmacodynamics. Saliva samples were collected using the Cozart RapiScan collection pad and tube and analyzed using the Cozart RapiScan Saliva Drugs Test (Cozart Bioscience). Saliva samples were obtained and analyzed at 0, 15, 30, and 60 minutes and 4, 6, 8, and 24 hours after drug administration. All salivary samples obtained within the first 9 hours after codeine administration were positive for opiates in all 5 subjects. Forty-three drug-free volunteers were similarly screened and used as controls. Forty-two of these subjects produced samples that were negative for the presence of codeine. One positive result was obtained in this control group from a patient who was taking prescribed ibuprofen and codeine and had neglected to mention this to the investigators.

Nicotine (cotinine). Saliva can be used to monitor tobacco smoking and exposure to tobacco smoke. The major metabolite of nicotine, cotinine, has been investigated as an indicator of exposure to tobacco smoke. Cotinine is tobacco-specific and has a relatively long half-life (17 hours) compared with nicotine.[56] Cotinine appears rapidly in saliva and plasma after nicotine administration, and saliva concentrations generally exceed corresponding plasma concentrations.[57]

Salivary cotinine levels in hospitality workers employed in establishments that permitted smoking were significantly higher than those sampled from employees of nonsmoking establishments.[58] Monitoring levels of salivary cotinine has proven useful in monitoring self-reported compliance with smoking cessation programs.[59,60]

Infectious Diseases

Specific antibodies have been detected in saliva and oral transudate to monitor a variety of infectious viral, bacterial, fungal, and parasitic diseases. Immunoglobulins gain entry to the oral cavity primarily through secretion from the salivary glands or transudation from the blood capillaries into the gingival crevicular fluid (GCF).[61] The predominant immunoglobulin in saliva, secretory IgA, is derived from plasma cells in the salivary glands and constitutes the main specific immune defense mechanism in saliva. Cells in the parotid and submandibular glands are responsible for the majority of the IgA found in saliva.[62] Conversely, IgG and IgM in saliva are derived mainly from serum via mucosal transudate and GCF and can be regarded as a dilution of the subject's plasma.[58]

Human immunodeficiency virus. Studies have demonstrated that the diagnosis of infection with the human immunodeficiency virus (HIV), based on specific antibodies present in mucosal transudate, is equivalent to serum in efficacy.[63] Compared with serum, the sensitivity and specificity of antibody to HIV in mucosal transudate for detection of infection are between 99.2% and 100%[64] when using OraSure (Orasure Technologies). OraSure is the only FDA-approved, commercially available testing system that can be used for the diagnosis of HIV-1 and HIV-2. The test relies on the collection of an oral mucosal transudate containing IgG antibodies via the placement of a collection device in the mandibular vestibule for 2 to 5 minutes. The sample is initially screened using an enzyme-linked immunosorbent assay (ELISA) assay, and positive diagnoses are confirmed via Western blot assay.

Analysis of antibody in saliva and oral transudate as a diagnostic test for HIV (or other infections) offers several distinctive advantages when compared with serum. The noninvasive nature in which a sample is collected eliminates the risk of infection inherent in collecting blood samples. Saliva and oral fluid have been shown to inactivate HIV and possess less infectious virus than blood.[51] Saliva collection also simplifies the diagnostic process in special populations whose blood is more difficult to obtain, including individuals with compromised venous access (intravenous drug users), hemophiliacs, children, obese people, and the elderly and infirm.

Hepatitis. Acute hepatitis A virus (HAV) and hepatitis B virus (HBV) have been diagnosed based on the presence of specific IgM antibodies in saliva.[65] Saliva has also been utilized to detect very low levels of antibodies to HAV associated with vaccine-induced immunity. Comparison of serum and saliva levels of infection and vaccine-induced HAV-specific IgG have demonstrated excellent agreement (sensitivity = 98.7%, specificity = 99.6%).[66]

Analysis of oral fluid samples collected with Orasure provided an excellent method for the diagnosis of HBV and hepatitis C virus (HCV).[67] In comparing oral fluids to serum, sensitivity and specificity of 100% for the detection of HB surface antigen and antibodies to HCV, respectively, were reported.[63,68]

Measles, mumps, rubella. Saliva may also be used for determining immunization to or infection with measles, mumps, and rubella.[69,70] The detection of antibodies in oral fluid samples from subjects having received a measles/mumps/rubella vaccine produced sensitivity and specificity of 97% and 100% for measles, 94% and 94% for mumps, and 98% and 98% for rubella, respectively, in comparison with detection of serum antibodies for these viruses.[66,69]

Vyse et al[71] conducted a study to compare 3 different saliva collection systems: OraSure (OraSure Technologies), Omni-SAL (Saliva Diagnostic Systems), and Oracol (Malvern Medical Developments). The aim of the study was to assess the specificity of oral fluid in

detecting the presence of antibodies to rubella and parvovirus B19 and to assess the reported acceptability to participants. One hundred forty-three children were randomized to use 1 of the 3 oral fluid collection devices. Oral fluid samples were obtained by a parent, who then completed a questionnaire recording information on ease of use and willingness to use the device again. Nursing personnel collected venous blood samples for comparison. These results supported previous studies in concluding that collected oral fluid is a suitable substitute for serum for surveillance and epidemiology. However, it was noted that the samples were not sufficient to give a reliable quantitative result. The proportion of parents indicating that they found the collection system quite easy or very easy to use was 98% for Oracol, 95% for OraSure, and 60% for Omni-SAL. The proportion who indicated they would probably or definitely not take another test was 0% for Oracol, 4.7% for OraSure, and 14% for Omni-SAL.

Cancer

The increasing use of biomarkers to assess systemic health and disease status has led to study of cancer markers in saliva. Recently published data indicate that the mRNA levels for specific proteins are elevated in the saliva of head and neck cancer patients, compared to healthy control subjects.[72,73] Combined data from 2 studies using RNA microarray followed by quantitative polymerase chain reaction (PCR) of candidate genes indicate that mRNA levels for interleukin-8, interleukin-1β, dual-specificity phosphatase I, spermidine/spermine N1-acetyltransferase, ornithine decarboxylase I, S100 calcium binding protein P, and H3 histone family 3A are elevated and the combination of these markers yields high sensitivity (91%) and specificity (91%) in distinguishing oral squamous cell carcinoma patients from controls.[73,74] Other studies have suggested that measurement of antibodies using ELISA to tumor-suppressor protein p53 in saliva may also be useful to detect those who suffer from an oral cancer lesion. The premise is that tumors with high levels of p53 elicit an immune response that results in the production of IgG and/or IgA antibodies to p53. The potential for specific mutations in the p53 DNA sequence being identifiable in saliva of head and neck cancer patients was first proposed by Boyle et al.[75] Following on, using PCR followed by DNA sequence analysis on DNA extracted from saliva, Liao et al identified mutation of the p53 gene at codon 63 as a molecular marker for oral squamous cell carcinoma.[76] Together, these preliminary data suggest that RNA, DNA, and/or antibodies can be used to detect markers for head and neck cancer.

Saliva has also drawn interest as a fluid carrier of the breast cancer proto-oncogene marker c-erbB-2.[77] The marker c-erbB-2 (also known as Her-2/neu) is often found in higher levels in malignant breast tumors, and tracking of response to therapy may include assessing whether c-erbB-2 levels have dropped at tumor sites. A circulating receptor of c-erbB-2 is found in serum and appears to be elevated in patients with carcinoma of the breast as well as being associated with occurrence of metastases. In a relatively small group of 25 women undergoing therapy for breast cancer of varying stages, salivary c-erbB-2 levels dropped significantly after administration of chemotherapy and/or radiation therapy, and the change mirrored that of serum c-erbB-2 levels.[78] These preliminary data indicate that saliva may carry markers of head and neck cancer as well as cancers from more distant sites and may be prognosticators of disease remission. Further research will identify which of these sample forms and which techniques offer the best opportunity to take advantage of the ease and efficiency of saliva collection.

Current National Initiatives for Salivary Diagnostics

From 2001 through 2004 the National Institute for Dental and Craniofacial Research invested 52 million dollars in salivary diagnostic research to concurrently spearhead the development of technologies to detect virtually any analyte in saliva as well as to comprehensively identify all the proteins in saliva. The emerging field of microtechnology- and nanotechnology-based biosensors will overcome the sensitivity barrier necessary to detect analytes in saliva. In addition, the salivary proteome will be comprehensively deciphered, catalogued, annotated, and available to the scientific community. Great progress is already on the way with the identification of 310 salivary proteins (www.hspp.ucla.edu). It is envisioned that with the human salivary proteome available, one can begin to exam and compare salivary proteomes of high impact diseases such as Sjögren Syndrome, osteoporosis, rheumatoid arthritis, diabetes, and cancers.

Caveats

Human saliva contains a panoply of proteins (antibodies), RNA, and DNA, which offer a significant potential to clinicians and patients who wish to take advantage of the ease and efficiency of saliva collection to detect either diagnostic or prognostic markers of disease. The potential, however, must be considered with respect for saliva's unique limitations. Clearly, collecting whole saliva is the most convenient method, but different methods (drooling, spitting, and use of absorbent material such as cotton) need to be definitively determined. However, whole saliva will also be "contaminated" with other biologic materials previously described.

Other issues that need investigation include whether collected saliva should be unstimulated or stimulated, and if the latter, what is the best way of stimulation. Certain salivary proteins display diurnal variability; therefore, each protein that is tested for diagnostic purposes in saliva must be evaluated to determine whether its concentration or presence is dependent upon this form of biologic variability. In addition, the effect of different handling protocols of saliva immediately after collection is crucial, because certain proteins are more stable at room temperature or through multiple freeze-thaw cycles than other proteins. Also, careful handling of saliva is required to ensure optimal protection of RNA and DNA. Saliva contains a significant level of mucins. These mucins, along with the chemical milieu of saliva, predispose to the formation of protein complexes, some of which lend themselves neither to easy disruption nor to easy determination of protein level by conventional assays such as ELISA, radioimmunoassay, or other standard means of proteomic analysis. Therefore, much work needs to be done to determine the most appropriate methods to process saliva prior to analysis.

At one extreme, it is likely that certain markers will not be assessable because of the limitations of saliva collection and analysis, while at the other extreme, some proteins will lend themselves well to easy-to-use assays conducted with whole unstimulated saliva that provide consistent results. In Part 2 of this review, we will discuss the potential for saliva to be used to measure bone turnover and present some preliminary results suggesting that 2 markers of bone turnover can be detected in whole unstimulated saliva processed in an uncomplicated manner using conventional commercially available ELISAs.

Summary

While salivary diagnostics are a historical concept, the current national investment and the scientific talents of the oral health community, accompanied by a host of emerging technologies, uniquely position saliva diagnostics to fully attain its envisioned goal for disease diagnostics as well as for normal health surveillance. Microtechnology- and nanotechnology-based biosensors will overcome the detection barriers, while the salivary proteome will provide the targets and roadmap for disease diagnostics. It is almost certain that the next few years will witness an evolving spectrum of salivary screening, and diagnostics applications via reference labs, point-of-care detections, or even home testing kits will begin to appear.

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Literature Abstract

Preventing and detecting oral cancer. Oral health care providers' readiness to provide health behavior counseling and oral cancer examinations

The purpose of this survey was to examine the oral cancer prevention and early detection practice patterns of a sample of practicing dentists (n = 1025) and hygienists (n = 1025) in the state of New York. The sampling was a population-based, self-weighting, stratified random sample from the roster of licensed practitioners in New York. A total of 904 dentists and 963 hygienists were eligible and sent surveys. The Tailored Design survey protocol was followed. A response rate of 55% for dentists and 66% for hygienists was obtained. The Transtheoretical Model of Change (TTM) was use to classify the oral healthcare providers readiness to conduct oral cancer examinations and to offer tobacco-use cessation and alcohol-abuse counseling. The TTM categories include: precontemplation, contemplation, preparation, action, and maintenance. Survey results found 82% of dentists and 72% of hygienists in the maintenance stage regarding their readiness to perform routine oral cancer examinations in their practices. Only 12% of dentists and 21% of hygienists were in the maintenance stage in terms of readiness to offer tobacco-use cessation counseling, with percentages decreasing to 2% of dentists and 4% of hygienists ready to offer alcohol-abuse counseling in the maintenance stage. Results confirm that oral cancer examinations are a standard of practice for oral healthcare professionals in New York state. Unfortunately, cancer prevention services, such as counseling regarding cessation of tobacco use and alcohol abuse, by oral healthcare professionals remain limited.

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