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Saliva and Hormones: Valuable Tool for Research and Diagnosis

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ABSTRACT

Saliva is a special characteristic of oral fluid and a biological specimen that may threaten measurement validity. Human saliva can be easily obtained by noninvasive techniques and contains many analytes of interest for screening, diagnosis and monitoring. These include steroid and other nonpeptide hormones, therapeutic drugs, drugs of abuse and antibodies. Numerous studies in the past 40 years have shown correlations between serum and saliva levels. Both diurnal and monthly profiles of hormone levels parallel traditional serum patterns. Multiple specimens for steroid hormone analysis can be easily collected by the patient, at home, to monitor fertility cycles, menopausal fluctuations, stress and other diurnal variations. Drug doses can be monitored without inconvenient and costly visits to blood-drawing facilities. Antibody levels can be determined to screen for infectious diseases. Saliva can be collected directly by spitting into a tube or with one of several devices, each of which has its own special advantages and disadvantages. Saliva can be used as a diagnostic specimen not only to obtain information more inexpensively and efficiently than serum, but also to provide information not readily available from serum testing.

Keywords: Saliva, hormones, saliva collection.

INTRODUCTION

More than 50 years of interdisciplinary study reveals that hormones influence, and are affected by, behavior. These reciprocal effects are highly dependent on social context.[1] Hormones have been used as markers of status and change in various physiological systems. Contemporary behavioral endocrinology research is focused on linking individual differences in the status and change in these systems with a variety of issues related to health, development, and psychosocial adjustment. Monitoring hormones noninvasively has advanced this agenda by enabling the study of variation in endocrine function in the context of everyday life. The attention saliva has received as a research specimen is due to the perception that sample collection is quick, uncomplicated, and minimally invasive and salivary assays are reliable and accurate. In many circumstances, however, specialized issues threaten the measurement validity of salivary hormones. This review mainly highlights several of these special issues in an effort to inform the most recent movement to develop diagnostic applications for oral fluids.

SALIVA COLLECTION FOR THE YOUNG AND OLD: Challenging task

Saliva collection from preterm newborns, infants in neonatal intensive care units, and healthy infants less than 3 months of age, is especially challenging.[2] After repeated and

sustained effort, the result is often insufficient. On the other hand, collecting saliva from the oldest-old can be time consuming and have a high failure rate. In a study of the oldest-old (73% older than 85 years), one-third of attempts failed to provide valid saliva samples. Xerostomia (dry mouth) affected more than half of the study's participants. Use of medications with direct, or iatrogenic, diuretic effects was high for those who had difficulty donating specimens.[3] Using saliva, as opposed to urine or blood, enables sample collection in special populations and circumstances. However, investigators must be aware that this perception does not match reality in some unique person-by-situation circumstances. Specimen collection failure yields missing data, and these findings highlight that "missingness" may be related to age, physiological states (xerostomia), ethnic and poverty status, as well as medications.[4] For diagnostic applications, inadequate sample test volumes negate many of the advantages of monitoring hormones in oral fluids and translate directly into missed opportunity, wasted time and resources.

EFFECTS OF SALIVA COLLECTION TECHNIQUES ON SALIVARY HORMONES

Historically, saliva collection devices involve cotton-absorbent materials placed under the tongue (2–3 min), cotton saturates with saliva. Then saliva is expressed into collection vials by centrifugation or compression. Most of the time, this approach is convenient, simple, and time efficient. However, when the absorbent capacity is large and sample volume small, the specimen absorbed can be diffusely distributed and recovery becomes problematic. Recoveries for the Salivette cotton pledget are only 38, 59, 74, and 83%, and for braided cotton rope only 15, 31, 64, and 74% when initial sample volumes were 0.25, 0.50, 1.0, and 1.5 mL. Poor saliva volume recovery has been associated with artificially low hormone estimates.[5,6,7] The process of absorbing saliva with cotton also interferes with immunoassay performance for testosterone, SIgA, dehydroepiandrosterone (DHEA), estradiol, progesterone, 17-OH Progesterone, and androstenedione.[8] Thus, the use of cotton-based material to collect salivary hormone data for research or diagnostic applications raises unique concerns. Early literature on salivary hormones addressed low specimen volumes by "simply" stimulating saliva flow by chewing (gums, dental wax) or tasting (sugar crystals, powdered drink mixes, citric acid drops), or smelling a variety of substances (e.g., orange oil). When used more than minimally and/or inconsistently, some of these methods affect immunoassay performance. Saliva is poorly buffered and oral substances containing acids easily lower salivary pH. As pH declines, antibody–antigen binding is compromised and artificially high salivary hormone estimates may result.[9] Stimulants also influence measurement of the levels of analytes that are dependent on saliva flow rate. To minimize this validity threat, the immunodiagnostic industry has reduced saliva test volume requirements from >200 µL (prior to 1998) to < 25µL and diluents now normalize sample pH or flag samples with pH problems. The need to stimulate saliva is obsolete. The risks of doing so for oral diagnostics far outweigh the benefit. Behavioral science champions measurement of multiple endocrine markers in contemporary biosocial models of individual differences. To "multiplex" from a single sample, collection must minimize interference across different assay protocols. To achieve this aim, we ask participants to imagine they are chewing their favorite food, slowly move their jaws as if chewing, and to let saliva pool in their mouth without swallowing. Specimen is gently forced through a short drinking straw into a storage vial. The procedure's advantages include the following: (1) a large sample (1–5 mL) volume can be collected within 5 min; (2) sample can be assayed for multiple markers; and (3) samples can be archived for use in

future assays. The disadvantage is that the procedure requires a competent, aware (and awake), and compliant sample donor. In situations that lack this type of donor, it is essential to have a material to absorb saliva from the mouth. Collecting saliva on filter paper may be a viable option. Dombrowski and colleagues sampled newborns by placing filter paper on the anterior portion of the tongue and holding it in place until "sufficient" saliva was obtained. Despite standardizing the time of exposure in the mouth, there is considerable range in the volume of saliva collected between individuals, and saliva collected using filter paper returned at least fivefold higher cortisol estimates than passive drool.[10] More recently, Neu and colleagues describe a filter paper collection procedure that has acceptable recovery and linearity. They report that saliva collection was very efficient, and only 2% of the samples from newborns were lost due to inadequate test volumes. When it is not possible to gather samples by other means, filter paper may be viable for some salivary hormones.

Granger and colleagues evaluated hydrocellulose microsponge devices (BD Opthlamatic, Walton, MA) as an alternative saliva collection technique. Microsponges are used during ocular surgery to collect very small volumes of liquid (tears).[11,12]The BD microsponge reaches its maximum capacity (~300 μL saliva) within 60 seconds. The test volume for most salivary assays (25–50 μL) was obtained within 20–30 seconds.[11] Sample recovery under conditions designed to represent very small volume availability is also adequate. Percent recovery from the microsponge device was superior to cotton-based materials especially when sample volumes were below 100 μL. The small size also allows collection of sample from sleeping infants without waking them. For infants (5–18 months of age), a 1-min collection period and the use of two sponges per collection maximized success of collecting sufficient volume.[4] The microsponge is appropriate for use with samples to be assayed for cortisol, cotinine, and salivary alpha-amylase but is not appropriate for dehydroepiandrosterone (DHEA) or testosterone.[11] In summary, each saliva collection approach is associated with a unique set of benefits and risks that prevent universal application. Specifying the manner that samples are collected, stored, and prepared for each analyte will be critical to ensure the future of oral diagnostics.

BLOOD "CONTAMINATION" AND SALIVARY HORMONES

Blood can leak into oral fluids due to injury (burns, abrasions, or cuts to the cheek, tongue, or gums). Blood in oral fluid is more prevalent for individuals suffering from poor oral health (i.e., open sores, periodontal disease, gingivitis), during the course of certain infectious diseases (e.g., HIV), and for those who routinely engage in behavior known to influence oral health negatively (e.g., tobacco use). Epidemiologic studies are equivocal that poor oral health is most prevalent among minority, lower socioeconomic status, rural, and third world populations. Blood leakage into saliva is more likely during teething and when shedding teeth. To meaningfully index endocrine function, quantitative estimates of hormone levels in saliva must be highly correlated with the levels measured in serum. This correlation depends on consistency in the processes that move circulating hormones into oral fluids. When the integrity of this process is compromised, the level of the serological marker (hormone) in saliva should be affected.[13]The impact of this phenomenon for oral diagnostics has received only scant empirical attention. Utilizing salivary transferrin as a surrogate marker of blood contamination, we studied the degree of contamination needed to affect salivary hormone levels and whether the effects are long- (30–45 min) or short-lived (10– 15 min).[14] We found that the effects depend on which hormone is assessed. Transferrin levels in saliva are positively associated with salivary DHEA, cortisol, and

testosterone, but explain less than 5% of the variance, and less than 0.1% of statistical outliers (+2.5 SDs) in salivary hormone distributions are due to blood contamination.[15,16] Field studies also show that even in children at high risk for oral health problems, blood contamination in saliva is very infrequent. Finally, the findings demonstrate that blood contamination is a characteristic of individual specimens rather than of individual donors. The presence of blood in oral fluids has the potential, albeit small, to introduce error variance in the levels of hormones measured in saliva. Sample donors should be screened for events in their recent history that could cause blood leakage into saliva by asking questions related to oral health, teething, shedding teeth, open sores, and injury. Sampling saliva for diagnostic use within 45 min of micro injury to the oral cavity should be avoided. Samples should be systematically inspected at the collection point and if visibly contaminated with blood, excluded from analyses.[15]

MEDICATION USE AND SALIVARY HORMONE

Using saliva as a specimen enables measurement of individual differences and intra-individual change in endocrine function in the context of everyday life. Advances in modern medicine have made over-the-counter and prescription medication use a common feature of most social worlds, and highly salient for those suffering from physical or mental illness. Knowing whether medications influence the measurement of salivary hormone levels seems imperative. Medications that dehydrate and reduce salivary flow and, simultaneously, have the potential to act directly on the function of endocrine tissues are of obvious concern (e.g., contraceptives, glucocorticoid containing medicines). Medications that alter subjective psychological experiences (e.g., antidepressants, narcotics) may indirectly influence salivary hormones by changing the impact of environmental events (e.g., stress) on endocrine activity. Other medications may affect movement of small molecules into oral fluids (e.g., blood thinners, vasoconstrictors), or leave residue that interferes with salivary assay function (e.g., teething gels). Finally, the most prevalent medications require examination. In three recent studies, we have addressed some of these possibilities. The effects of exogenous glucocorticoid use on salivary cortisol measurements were dependent on both the type and amount of inhaled/nasal steroid used.[17] Compared to infants not taking any medications, stress-related cortisol reactivity was less pronounced for those taking acetaminophen.[18] Also, cortisol levels were higher for mothers taking oral or transdermal contraceptives and acetylsalicylic acid (e.g., Aspirin) but lower for mothers taking pure agonist opioids (e.g., Oxycontin) compared with mothers not taking medications.[18] Relative to a no-medication comparison group, children taking antipsychotic medications had higher DHEA levels and flat cortisol diurnal rhythms, (2) Ritalin or Adderall had flat testosterone diurnal rhythms, (3) Concerta had higher testosterone levels, (4) antidepressants had flat DHEA diurnal rhythms, and (5) hypotensives had flat cortisol and DHEA diurnal rhythms and higher testosterone levels.[19] A variety of medications are capable of introducing variance in salivary hormones. Medication use must be considered when interpreting the meaning of individual differences in salivary hormones in any research, screening, or diagnostic application.

BACTERIA AND SALIVARY HORMONES

Saliva specimens are often gathered in conditions that restrict how they can be handled and stored. Typically, once specimens are collected, samples are kept cold or frozen to maintain sample integrity. Refrigeration prevents degradation of some salivary hormones, restricts activity of proteolytic enzymes and growth of bacteria.

For large-scale national surveys, investigators working in remote areas, or patients collecting samples at home, freezing and shipping samples can be cost-prohibitive. In the past, to preserve a sample when refrigeration is not possible, sodium azide (NaN3) has been added. Disappointingly, NaN3 interferes with the activity of horseradish peroxidase in enzyme-based saliva immunoassays. The impact of bacteria-related issues on the measurement of salivary hormones by immunoassay has recently been explored. There are significant declines in the levels of some salivary hormones when samples are stored at RT or 4◦C in comparison to −60◦C after 96 h.[20] Attempts to minimize this phenomenon by filter-sterilization were largely unsuccessful because passing saliva through 0.22-μm pores was impractical.[21] Subsequently, we treated saliva with penicillin/streptomycin to inhibit bacteria growth. Samples were treated either a prior (immediately after collection and then left at RT) or post hoc (samples were collected and then stored at RT prior to treatment). Within 48 h at RT, salivary alpha-amylase (sAA) and salivary cortisol levels had declined by 34 and 5%, respectively. The negative effect of RT storage on sAA and cortisol was minimized by a priori, but not post hoc, treatment with Pen-Strep. There are considerable individual differences in the quality of saliva that may interact with how samples are handled, stored, and transported after collection. When these potential sources of error variance remain unaccounted for researchers, medical professionals, and patients should question specimen integrity and any conclusions drawn from the measurement of salivary hormones.

CONCLUSION

More than 25 years of research involving the measurement of hormones in saliva reveal that there are wide-ranging individual differences between individuals and within individuals over time in the levels of most salivary hormones. Cross-study consistency links salivary hormones to social forces, specific behaviors, developmental trajectories, or health status. We expect drawing attention to these issues will increase the probability that salivary hormones will be more successfully integrated into the next generation of biobehavioral research and, in doing so, set a more solid foundation for the eventual translation of the basic findings into clinical investigation and diagnostics.

Declaration by Authors

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