
SALIVA IS A VALUABLE DIAGNOSTIC TOOL FOR HORMONE ASSESSMENT

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The use of saliva as a vehicle for determination of plasma steroid hormone levels has increased dramatically in recent years. Since 1983, more than 2500 papers and research articles dealing with salivary diagnostic tests have been published¹. Both clinicians and investigators have used saliva to assess numerous clinical problems including, digitalis toxicity, celiac disease, liver function and immunodeficiency². Saliva has also been used for pharmacokinetic studies and therapeutic drug monitoring in a variety of clinical situations. The value of saliva as a monitoring medium resides in the fact that it is easy to collect, store and ship, and is non-invasive, thus convenient for multiple sampling.

PLASMA-SALIVA TRANSFER

The reliability of saliva testing depends on establishing a direct correlation between saliva and plasma concentration of a particular substance. The transfer of substances from plasma into the saliva is dependent on their physicochemical properties¹. A small molecular weight and a great lipid solubility are normally associated with a faster transfer rate. A good correlation has also been established between saliva/plasma ratio of substances, their pKa and salivary pH. Salivary flow rate and the existing pathophysiology of the oral cavity have also been shown to affect salivary distribution of substances.

Saliva concentration of a particular hormone is dependent on the affinity and total binding capacity of various binding proteins in plasma.

As blood passes through salivary glands, free "unbound" and weakly bound (low affinity binding protein) forms of hormones will diffuse through the salivary gland epithelium into the saliva. As in other clearing organs, membrane transfer occurs in both directions, is passive for most substances and equilibrium is governed by the transmembrane concentration gradient. Thus, saliva levels reflect the free concentration of hormones in plasma, and in the absence of high affinity, high capacity-binding proteins, these levels correlated with plasma concentrations. On the other hand, hormones that have high affinity, high capacity-binding proteins such as thyroxine, are difficult to assay in the saliva. These hormones have a very high plasma total to free hormone ratio and exist in small amounts in saliva.

SALIVARY STEROID HORMONE ANALYSIS

Monitoring plasma steroid levels is essential for the clinical assessment of a patient's endocrine function. Saliva becomes an important diagnostic tool, since in many instances, the standard plasma and urine sampling techniques may not provide the optimum sampling conditions. Some of the problems that diagnostic laboratories had to overcome in establishing the validity of salivary steroid assays, were to determine whether steroid concentration in saliva can be measured with accuracy and whether these small values are meaningful and correlate with plasma levels or any other physiological parameter.

Salivary steroid levels tend to be much lower than those in plasma, because they reflect the level of unbound steroid, which represents about 2-5% of total plasma concentration. Salivary glands may also transform certain steroids during their passage across the salivary epithelium³. Highly polar molecules, such as sugars and conjugated steroids do not cross the lipid lining of cells and can only get into whole saliva via blood contamination from small cuts and ulcers from gingival fluid.

Here we review the pharmacodynamics and partitioning of several hormones, that have been characterized in saliva and evaluate the correlations between their saliva and plasma concentrations.

PROGESTERONE

Progesterone was one of the first steroids to be reliably assayed in human saliva. Once saliva samples are collected, salivary progesterone concentrations remain stable under a wide range of handling conditions. Because of its high circulating blood levels (ng/ml during luteal phase), saliva concentrations of the hormone usually remain within the limits of sensitivity of most conventional assays. Average salivary progesterone concentrations vary from 20-100 pg/ml during the follicular phase to 100-500 pg/ml during the luteal phase (Figure 1). Progesterone levels are also affected by women's age, degree of activity, nutrition and race^{4,5}.

A high correlation coefficient (between 0.8 and 1.0) has been established between plasma and salivary levels of the hormone^{5,6} making saliva a useful diagnostic tool. Serial measurement of salivary progesterone has been used to assess ovarian function, and in particular for diagnosis of defective or inadequate luteal function, as well as to monitor response to hormone therapy. Other clinical applications may include monitoring placental function by repetitive measurement of salivary progesterone during pregnancy⁹.

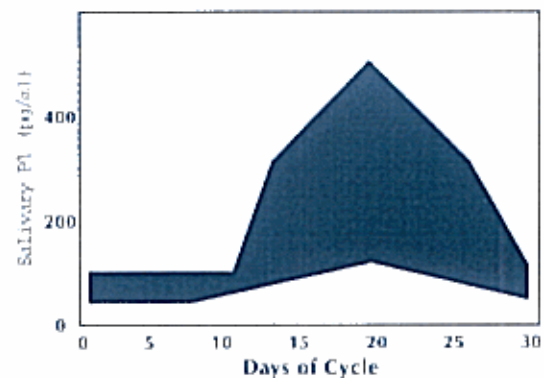


Figure 1: Range of salivary progesterone concentrations during a normal unstimulated menstrual cycle.

ESTRADIOL

Salivary estradiol (E_2) is about 1-2% of total plasma values. Earlier studies, using assays with variable sensitivities and specificities, have described a wide range of salivary estradiol levels. Three independent studies using radioimmunoassay¹⁰⁻¹², enzyme immunoassay¹³ and chemi-luminescence immunoassay¹⁴ have reported comparable salivary E_2 levels during normal non-stimulated menstrual cycles ranging from 5-15, 10-30 and 7-20 pmo/L during the follicular, periovulatory and luteal phase, respectively (Figure 2). These levels in stimulated cycles ranged from 10 to 120 pmo/L¹⁵.

Moreover, Wong et al.¹⁰ showed a mid-cycle salivary E_2 peak, corresponding to the mid-cycle LH surge, followed by a midluteal rise. In these women, the changes in salivary E_2 were similar to the E_2 pattern in serum, with a high degree of correlation ($r=0.93$). A similar correlation ($r=0.96$) was observed between salivary E_2 + estrone and free plasma E_2 in FSH-stimulated cycles¹³.

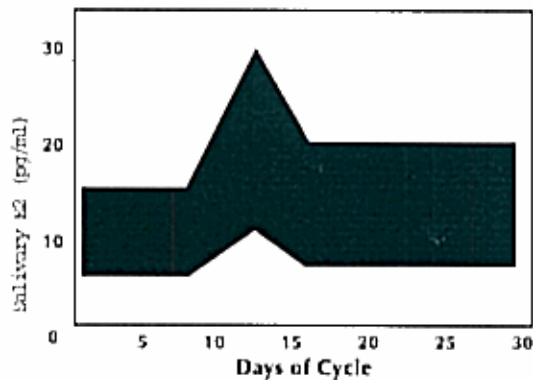


Figure 2: Range of salivary estradiol concentrations during a normal unstimulated menstrual cycle.

TESTOSTERONE

Under physiological conditions, a very good correlation exists between salivary and serum testosterone (T) in men¹⁶⁻¹⁷. Wang et al. have demonstrated that following exogenous T administration, salivary T rose in parallel with total serum T, without significant changes in serum sex binding globulin (SSBG)-binding capacity. This observation strongly suggests that salivary T represents the non-protein bound fraction of the hormone in females, salivary testosterone also showed a close correlation with free unbound plasma testosterone when calculated from the measurement of sex steroid binding globulins-binding capacity in normal females and patients with polycystic ovarian disease¹⁵.

Salivary testosterone levels in adult males have been reported to range between 150-500 pmo/L¹⁷⁻¹⁸, with significantly lower levels reported in females (<50 pmo/L)¹⁸⁻¹⁹. Testosterone concentrations are known to follow a diurnal patterns and are affected by a number of factors including age²⁰ and activity²¹.

CORTISOL

Salivary cortisol measurement is a practical and convenient approach to assessing pituitary adrenal function^{22,23}. Unbound, biologically active cortisol enters saliva predominantly transcellularly, independent of saliva flow rate^{22,24} and closely reflects the concentration of free cortisol in blood^{22,25}. Clinical studies²⁶

have demonstrated a close parallelism between the changing profile of plasma total and salivary cortisol concentrations under a variety of stimulation and suppression tests used to investigate the patency of hypostalamopituitary-adrenal (HPA) axis. A close correspondence in circadian and ultradian fluctuations in salivary and plasma cortisol was observed²⁷. Tunn et al.²⁸ also showed that in corticoid-suppressed volunteers, both saliva and serum cortisol concentrations showed a nearly identical time course following intravenous administration of cortisol. Cortisol appeared in saliva 5 min after an increase in serum cortisol.

The convenience of salivary cortisol testing is that it allows multiple sample collections in a stress-free environment, while the patient maintains normal daily activity. It also provides a means to study the effect of alteration in activity/rest schedules and stress on adrenal activity. Studies have shown both acute and strenuous exercise may cause a significant increase in both plasma and salivary cortisol^{29,30}. Exposure to stress also results in a severity-dependent rise in salivary cortisol³¹. Salivary cortisol has also been used to evaluate the HPA axis activity in patients with psychiatric disorders.

Salivary cortisol exhibits clear diurnal variation and circadian rhythmicity both in normal and depressed individuals. Salivary cortisol is highest in the morning and varies between 13 and 23 nmoles/L. These levels decrease significantly during the day and reach their lowest value at night (1-3 nmoles/L) (Figure 3).

DEHYDROEPIANDROSTENEDIONE (DHEA)

DHEA is an adrenal steroid produced in abundant amounts and is conjugated to sulfate to form DHEAS before its release into the circulation³². DHEA and DHEAS are interconvertible and exist in dynamic equilibrium with each other³³. Salivary DHEAS is found in saliva at about 0.1% of its plasma concentration. Serum fluctuations in DHEA(S) concentrations are accurately and rapidly reflected in salivary levels³⁴.

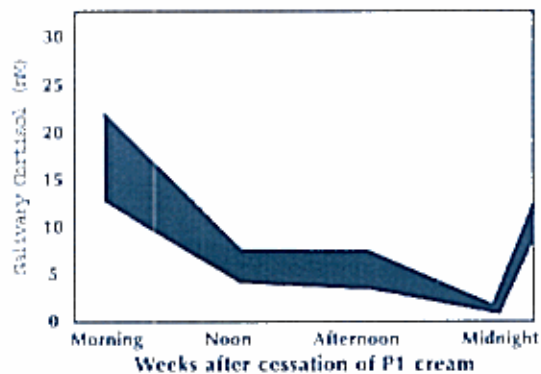


Figure 3:
Normal reference curve for diurnal adrenal profile.

OTHER HORMONES

Salivary estriol levels are also high, easy to measure and correlate well with plasma unbound unconjugated estriol^{35,36}. Androstenedione can also be measured with sufficient accuracy in saliva. The absence of specific high affinity binding proteins for androstenedione, results in a linear relationship between plasma total and plasma free fraction, and hence an excellent correlation between plasma and saliva levels of the hormone³⁷.

CONCLUSION

The above studies clearly demonstrate that saliva is a useful diagnostic tool for measurement of steroid hormones. Salivary concentration represents the free form of a particular hormone, and thus is a true reflection of its bioactivity. Moreover, the non-invasive nature of saliva collection and the convenience of multiple samples facilitate the design of functional assays for the assessment of various endocrine functions. In a recent review, Mandel³⁸ has likened saliva to a mirror reflecting the emotional, hormonal, immunological as well as nutritional and metabolic status of the body. The broad spectrum of interactions and relationships among these factors opens a whole field of diagnostic possibilities worth exploring and evaluating.

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