Steroid Analysis in Saliva: An overview

Abstract

The first report of steroid analysis in saliva was more than thirty years ago. Since that time its popularity has increased due to the attractiveness of non-invasive, repeated and simple stress-free sampling. It has proved a popular sampling fluid for psychobiology, sports medicine, pharmacology and paediatric studies as well as in the area of complementary medicine. In the diagnostic laboratory, salivary progesterone and oestradiol have been used for assessing ovarian function and 17α-OH progesterone for the diagnosis of congenital adrenal hyperplasia (CAH). Salivary cortisol is used for investigating adrenal function and recently there has been considerable interest in the use of bedtime salivary cortisol levels as a screening test for Cushing's disease. However, there are several caveats on the use of saliva including collection techniques, the variable matrix of saliva, sensitivity, steroid stability, the presence of binding proteins and reference range anomalies. This brief review will attempt to address these issues and provide a balanced approach to steroid analysis in saliva.

Saliva Collection, Storage and Pretreatment

There are several saliva collection techniques and devices available. Flavoured beverage crystals and lemon juice have been used to stimulate saliva flow. Flavoured crystals may cause a small increase in measured cortisol values whereas lemon juice has been reported to compromise the extraction of cortisol from saliva.[16,17] To maintain consistency their use is probably best avoided. Various absorbent devices have been used to facilitate saliva collection. They are rated acceptable and easy to use by participants.[18] These devices may be applicable for research with children and the elderly. However, there are reports that salivary cortisol values are reduced by more than 50% when saliva is not retrieved immediately from cotton buds.[19] Conversely, others have reported no effect for cortisol and artificially elevated results for testosterone, DHEA, progesterone and oestradiol using cotton absorbent materials.[20] There have also been several reports on the stability of steroids in saliva. Storing saliva samples at room temperature is not desirable as bacterial activity has been reported to be associated with changes in testosterone but not cortisol or DHEA.[21] Cortisol, 17α-OH progesterone and progesterone are reported to be stable after five days at 4° C in intact and centrifuged saliva and unaffected by two freeze /thaw cycles.[22] Similarly others have shown that cortisol is stable in centrifuged saliva for even longer periods at 5° C and up to three months at −20° C with notable losses occurring at room temperature.[23] Pretreatment of saliva may affect the concentrations of some steroid hormones when measured following extraction and chromatography. Sonication resulted in significantly higher saliva values than supernatant values for progesterone, cortisone, 17α-OH progesterone, testosterone and oestradiol. No differences were observed for cortisol and androstenedione.[24] Comparisons were benchmarked against levels measured following extraction and chromatography of the saliva samples. Taken together, the collection of unstimulated saliva into small plain tubes and storage at −20° C would appear to avoid any possible pitfalls. If long term

storage is anticipated then storage at −80° C is desirable. If special collection devices are to be used then ideally the same device should be used throughout a particular study, including the derivation of reference ranges. Furthermore, analysis of intact saliva, preferably following solvent extraction, is sound analytical practice.

Salivary Glucocorticoids

There is little information on 11-deoxycortisol in saliva, presumably due to its very low level, although it can be detected following metyrapone.[54] On the other hand, salivary cortisol determinations are more promising. Since the earliest reports, there is evidence that saliva levels reflect unbound concentrations in plasma.[55] Direct measurement in saliva may be comparable to extraction but extraction has the advantage of allowing the analysis of low volume saliva samples. This can avoid the use of stimulants and the loss of data due to insufficient sample volume.[56] Salivary cortisol determinations have proved popular in psychobiology, stress and sports medicine studies.[57–61] Their use is based on the assumption that salivary cortisol is a reasonable reflection of hypothalamicpituitary- adrenal (HPA) axis function. Indeed, in the diagnostic setting, salivary cortisol levels parallel those in plasma following ACTH and CRH stimulation, and following exercise induced-stress.[62,63] However, the correlation of salivary cortisol levels with total plasma cortisol is confounded by the presence of corticosteroid-binding globulin in plasma which is largely saturated up to 500–600 nmol/L of cortisol.[62] Salivary cortisol correlates better with measured plasma free cortisol than total plasma cortisol. However, it appears to be subject-specific as considerably variability is found between individuals for daily paired samples (Figure 2). Salivary cortisol determinations were used as markers of metabolic disturbances in obese and diabetic patients and used to investigate changes in glucocorticoid control of the HPA axis following oral prednisolone.[64–67] Conversely, salivary cortisol measurement is not a useful tool in determining dose adequacy in subjects on oral glucocorticoid replacement therapy.[68,69] Blood spots or serum is preferable, due to contamination of saliva by oral hydrocortisone. The diurnal variation of plasma cortisol is reflected by similar changes in salivary cortisol and hence timed salivary cortisols have been used in a diagnostic setting. Early morning salivary cortisols are useful as a screening tool for adrenal suppression and salivary cortisol is helpful to investigate the control of diurnal cortisol secretion following exposure to darkness and light.[70–72]

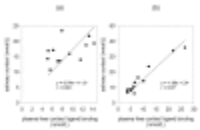

Figure 2
Correlation of salivary cortisol and plasma free cortisol in paired samples from two normal individuals (a and b). Salivary cortisol was measured by ELISA and plasma free cortisol by ligand binding/ultrafiltration.

Recently, there has been considerable interest in the use of night time salivary cortisols for the initial screening for Cushing's syndrome. However, despite the optimism, an element of caution

is required. Reported cut-off values differ considerably. Papanicolaou et al. determined a normal night time (2330–2400h) saliva cut-off of 15 nmol/L with values above this threshold suggestive of Cushing's.[73] They reported 100% specificity and 91% sensitivity for saliva, similar to a late night serum cortisol measurement cut-off value exceeding 240 nmol/L. Both were reportedly clearly superior to urinary free cortisol excretion, contrasting with other recent reports. Yaneva et al.[74] determined a lower saliva cut-off value of 5.7 nmol/L, with 100% sensitivity and 96% specificity which effectively separated Cushing's from obese subjects.[75] The corresponding excretion of urinary free cortisol determined a cut-off value of 248 nmol/day, with similar sensitivity and specificity to saliva. Viardot et al. reported a similar night time saliva cut-off value of 6.1 nmol/L and view salivary cortisol as a reliable first line alternative to urinary free cortisol, the urinary free cortisol:creatinine ratio, and the 1mg overnight dexamethasone suppression test.[75] However, their cut-off value for urinary free cortisol was considerably higher at 504 nmol/day compared to the 248 nmol/day reported by the Yaneva group.[74] Both groups used extraction based assays for urinary free cortisol which, as an aside, highlights the importance of well characterised methods and reagents for the determination of urinary free cortisol.

Late night salivary cortisol measurement is nevertheless very promising for the diagnosis of Cushing's although elevation above threshold values can occur in the elderly, diabetics and women in late pregnancy.[75,76] The variations in reported late night salivary cortisol cut-off values could result from the relatively small numbers in each of the study control groups, which may have varying degrees of obesity, non-adrenal disorders and pseudo-Cushing states, which themselves may be influenced by periodic hypercortisolism.[73] Methodological and standardisation issues are also likely contributors to differences in reported cut-off values but a major factor is probably differing specificities of cortisol antibodies towards cortisone. The salivary gland has abundant 11 β-hydroxysteroid dehydrogenase type 2 activity and as a consequence, saliva, unlike plasma, has up to three times the level of cortisone compared to cortisol.[14,77] Depending on the relative cross-reactivity of cortisol antibodies towards cortisone, there could be quite different values of salivary cortisol measured by different immunoassays. Conversely, differences in plasma would be expected to be minimal as cortisone levels are normally only 10% of circulating cortisol levels.[77] It is therefore desirable that laboratories establish their own method-specific reference ranges before using salivary cortisol for diagnostic purposes.

Conclusion

Salivary steroid testing has a recognised place in research and diagnostic medicine although its limitations must be acknowledged. It is clearly not desirable for androgen assays as well as assays to assess ovarian function and the monitoring of absorption of steroids from transdermal creams. Caution must be exercised for these applications. The use of salivary cortisol for measuring endogenous cortisol is the most encouraging. It can be successfully applied to research studies, adrenal stimulation tests, investigating diurnal variation as well as night time samples as a screening test for Cushing's syndrome. However, there is a need to establish methodspecific reference ranges and sample stimulation, collection and storage should remain consistent across study groups with a preference for the collection of whole unstimulated saliva, if possible.