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Review Article

Diagnostic usefulness of salivary reproductive hormones: An update

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Abstract

Of late research on the diagnostic use of saliva to measure various biochemical analytes, notably hormones are on the increase. The collection of saliva is a non invasive procedure and least uncomfortable to the patients. Special precautions are not required and large volume of specimens could be easily collected. Many studies have been carried out in this field as early as late 1960's. This review article brings out recent findings in the use of saliva to measure both male and female reproductive hormones, since the first line of laboratory tests to evaluate infertile couple start with reproductive hormones measurement. The important reproductive hormones measured in serum are total testosterone, free testosterone, Sex Hormone Binding Globulins (SHBG) for men and progesterone, oestrogen, oestrone, cortisol, 17-OHProgesterone for women. Many studies have established significant correlations between salivary and serum reproductive hormones and this article highlights the clinical usefulness of such hormones measured in saliva.

Keywords: Salivary Testosterone, Cortisol, Salivary Progesterone, Progesterone, Oestradiol

1. Introduction

Saliva, as a biological specimen has been in use since the late 1960 and recently numerous publications regarding the use of saliva to test biochemical substances, especially reproductive hormones for both males and females to diagnose hormonal imbalance as well as for the evaluation of Infertility have been published. The advantages of using saliva is that it is a simple non invasive procedure and samples could be easily collected at home and does not require needles. Hormones levels measured in saliva reflect the non protein bound free fraction of hormones, which are considered biologically active at a particular time of collection. Such free hormones level reflects the patient's hormone related problems. Further, most recent studies have proved that there is statistically significant correlation between salivary and serum hormone levels. Such measurements of hormones in saliva could be useful in the diagnosis of various disorders such as fatigue, weight gain, mood changes, and menstrual problems for women, pre menstrual symptoms, Poly Cystic Ovarian Syndrome (PCOS), insomnia, menopausal problems, stress, cravings, hormonal imbalances and flushing. Further, measurement of sex steroids both in men and women will help to diagnose reproductive issues, infertility and a wide range of related diseases. Almost all reproductive hormones such as Testosterone, Free Testosterone, SHBG, Dehydroepiandrosetrone-sulphate (DHEA-S) and Cortisol for men, and Oestrone, Oestradiol, Oestriol, Progesterone, Testosterone and DHEA-S for women could easily be measured using latest hormone testing procedures. This paper brings out the latest findings in the use of saliva to measure hormones related to infertility and its clinical usefulness.

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 collection facilitate the design of functional assays for the assessment of various endocrine functions[1]. Salivary testosterone (Sal-T) measurements could be useful in behavioral research, where subjects are often reluctant to provide serum samples. The usefulness of salivary measurements depends upon their reliability. Mean testosterone (T) concentration dropped about 50% from morning to evening for both sexes, with the largest drops early in the day. Salivary assays offer a practical way of measuring T in free-ranging subjects outside the laboratory[2].

Sal-T concentration is higher compared to the serum T concentrations in hirsute women may reflect the bioavailability of albumin-bound T or an ability of the salivary glands to metabolize steroids. Sal-T was more closely related to the T/SHBG ratio, reflecting the non-SHBG-bound fraction of T, than to serum Free Testosterone (FT), which might support the former theory. Sal-T correlated better to the degree of hirsutism than did any of the serum T parameters or SHBG. The correlation between Sal-T and hirsutism was partly dependent on the effect of body mass index. After eliminating this effect, Sal-T still correlated with hair growth on the total body area. On the basis of the results, Sal-T seems to relate to the bioavailable fraction of the hormone and, thus, appears to be an optimal method for studying hirsute women[3]. A major focus of testosterone analysis in saliva is in infertility research for the treatment of male hypogonadism. T measurement in saliva has been successfully used to differentiate between eugonadic and hypogonadic men. Sex-steroid binding globulins play only a minor role in saliva. Because of the close correlation of Sal-Tto serum FT, Sal-T concentration was established as a biomarker in the diagnosis of male androgen deficiency. Sal-T levels correlated positively with all circulating androgens, showing the best correlation with FT. A cut-off value of Sal-T< or = 0.195 nmol/L showed 100% sensitivity and specificity to rule out hypogonadism. In order to validate Sal-T, its reproducibility, the agreement with serum FT, the correlation with other circulating androgen markers (bioavailable T, total T) cut-off values should be defined. Sal-T is a reliable marker of testosterone bioavailability and the results support the inclusion of this biomarker as a noninvasive approach in the diagnosis of male androgen deficiency[4].

There are gender differences in Sal-T levels and variance, the serum-saliva association, the relationship of Sal-T to age and pubertal development, and the stability of individual differences in Sal-T levels over time and the findings have important implications at several levels of analysis for research that aims to test biosocial models of T behavior relationships. Recommendations are provided to steer investigators around

these "troubles" with Sal-T[5]. Sal-T provides a sensitive, simple, reliable, non-invasive and uncomplicated diagnostic approach for PCOS[6]. Salivary androstenedione/Sal-T ratio may be a good indicator of hyperandrogenism in women and measurement of androstenedione in plasma may be useful in making a diagnosis of PCOS[7]. Sal-T levels may be used for evaluation of androgen status in male infertility[8]. There were no significant differences in T levels compared with an age-matched control group. In the patient group, pre-dexamethasone levels correlated significantly and negatively with depression ratings on the 21-item Hamilton and the Montgomery and Asberg depression scales, and also with state anxiety measured on the Spielberger scale[9]. Higher T levels were found in male and female participants using selective serotonin reuptake inhibitors than in non-users. Sal-T levels are lower in female patients with a depressive disorder, generalized anxiety disorder, social phobia, and agoraphobia as compared to female controls. Selective serotonin reuptake inhibitors may increase Sal-T in men and women[10].

Clinical assessment of androgen action and its correlation to levels is a challenging job for clinicians. The current gold standard is measuring biologically active salivary FT as a possible substitute for serum Total T[11]. Changes in Sal-T, T, FT and SHBG concentrations did not show that changes in FT concentrations in serum of the groups studied, which is believed due to its lower specificity. Sal-T is a useful indicator for monitoring androgenic status in women with Rheumatic Arthritis[12]. Serum T and SHBG are relatively low in young women with premature ovarian failure (POF) and their Free Androgen Index (FAI) is therefore within the normal range. However, Sal-T, which measures FT, is consistently low to undetectable in these young women with POF. The reliability of the FAI as a marker of androgen deficiency remains questionable[13].

Measurement of the level of FT is important in the evaluation of testicular function. Because most of the testosterone in the saliva is in the free form, measurement of the Sal-T level is considered to be effective for the evaluation of testicular function. While the association of Sal-T to serum total T was at a significant level of $P<0.01$, its association to FT in serum was at a higher significant level of $P<0.001$. These findings thus indicate that the correlation between the Sal-T concentration and the serum FT concentration is better than that between the Sal-T concentration and the serum T concentration. Sal-T concentration decreased significantly with aging after the fifth decade of life. The correlation coefficient for that relationship was -0.606 ($p < 0.001$), and the change was similar to that seen in the serum FT concentration as a function of aging. The findings suggested that measurement of the Sal-T concentration is useful for the evaluation of testicular function[14].

A single i.m. injection of 50 mg T propionate to monkeys increased both Sal-T and T levels promptly and in parallel and the Sal-T levels correlated well with the serum T values and hence determination of Sal-T can be used as an index of free testosterone in this animal. Moreover, the monkey can be used as a model for studies in the human involving monitoring of salivary testosterone[15]. A significant correlation was observed between Sal-T and serum FT in matched serum and saliva samples over a wide range of concentrations. Sal-T measured offers a simple and cheaper alternative to serum FT measurement with the additional advantages of a stress-free non-invasive sampling procedure[16]. Free saliva testosterone (FSal-T), serum FT and LH/FSH and the FSal-T and LH/FSH ratio were significantly increased in patients with PCOS in comparison with the control group. Frequency of skin manifestations was significantly increased in PCOS patients with abnormal saliva and serum FT level in comparison with those of normal level hormones. There was a positive relationship between the increase in frequency of skin manifestations and increase in saliva and serum FT levels, while there was no relation between LH/FSH ratio and frequency of skin manifestations. FT level represents the most sensitive biochemical marker supporting the diagnosis of PCOS[17].

Sal-T concentrations in healthy men in morning hours were statistically significantly higher than that in men with androgen deficiency. Repetitive determination of FT concentrations in saliva (once a week for 5 weeks) showed high stability of results over time. FSal-T levels in morning samples correlated well with calculated free testosterone in serum, both in healthy men, and in patients with androgen deficiency, though in cases with very low T, salivary concentrations were systematically higher than calculated free testosterone levels in serum[18]. Serum and salivary concentrations of DHEA-S were significantly correlated and even under exercise conditions, the salivary values of cortisol and DHEA-S can reflect the behaviour of these hormones in serum. However, further studies are necessary to verify if Sal-T reflects the behaviour of serum FT during resistance exercise[19].

A successful pregnancy was seen only in association with consistently normal salivary progesterone (Sal-P) profiles or share the empirical use of clomiphene citrate therapy had corrected previously diagnosed luteal phase insufficiency. Basal body temperature records or mid-luteal serum progesterone (SP) measurements were less satisfactory indices of luteal function than Sal-P profile[20]. P levels remained abnormally high even when the SP cream was not being used as seen on day 2, reflecting carryover from the previous cycle and day 24, and showing carryover in the present cycle. Women using transdermal creams should be closely monitored for over dosage, and that perhaps the use of alternative modes of P administration should be considered, until more data characterizing the pharmacokinetics of P creams are available[21]. Low levels of P were detected in 50% of the male urine analysed. However, urine samples from men who had engaged in recent sexual activity contained relatively high concentrations of P which could be readily detected in 10 mL of urine. These results emphasise the potential of these compounds as specific and sensitive markers for the presence of human semen[22]. Sal-P profiles derived from daily sampling of 20 infertile women patients not only allowed accurate assessment of ovarian dysfunction but also indicated more effectively than conventional techniques the change in hormonal status following ovulation induction therapy with clomiphene citrate or bromocriptine[23].

P concentrations in saliva during the follicular phase of the cycle were low but raised beginning on day 12 to reach peak values on day 21. Thereafter, P concentrations in saliva declined at the commencement of menses[24]. The mean concentration of P in the follicular phase increased in the peri-ovulatory period to a peak 6 days following follicular rupture[25]. Sal-P measurements for diagnosis of corpus luteam function and highlight the difficulty of selecting representative reference populations in studies on female reproductive endocrinology[26]. A single mid-luteal Sal-P estimation or the mid-luteal LentonP index ($n = 4$) satisfactorily reflected the normal luteal phase, but a frequency of one sample every 3 days over the luteal phase ($n = 5$-6) was necessary to allow recognition of a short luteal phase or poor surge[27]. Sal-P concentrations in the luteal phase insufficiency group showed significantly lower values compared with those in the normal group between days 3 and 10. The cutoff values of 189 pmol/L in the midluteal phase yielded a sensitivity of 78.0% and a specificity of 76.5%, suggesting that daily Sal-P profiles during the luteal phase and a simple estimation of midluteal Sal-P appeared to be useful for the diagnosis of luteal phase defects[28]. Correlation between P concentrations in serum and in saliva is good.[29] Since there is a significant within-subject, between-cycle correlation in Sal-P index values, the clinical information derived from one cycle is likely to be representative for that individual[30].

Significant correlation was found between salivary and serum levels of estradiol (E2) and P. Measurements of these salivary steroids may be used to assess follicular dynamics[31]. In a study total or non-protein bound E2 levels measured in blood samples from normal women were both linearly correlated with the concentration of E2 in matched saliva samples. The amount of free oestradiol in blood was about twice that found in saliva[32]. The increase in saliva Oestriol (Sal-E3) with gestational age was consistent with the well established pattern for serum Oestriol (E3), with the median value exhibiting a small but significant rise between 32 and 33 weeks and a larger rise between 36 and 37 weeks. The ease with which saliva samples may be collected together with the high correlation between saliva and serum unconjugated E3 levels suggest that assay of sal-E3 should replace serum E3 measurement for assessing feto-placental wellbeing[33]. Simple, direct assays for Sal-P have been established, but those for E2 require considerably more research before becoming useful in routine practice. Predicting ovulation with data derived from saliva sampling awaits the development of more suitable assays for salivary estradiol (Sal-E2)[34].

Stress, as measured by saliva cortisol (Sal-C) and the Fertility problem inventory, does not negatively impact the effectiveness overstimulation and is not associated with a reduced number of oocytes[35]. Studies suggests that salivary measures represent the biologically active, free fraction of cortisol (C) and greater relative increase insal-C in response to tests that raise the absolute C concentration above the saturation point of Cortisol Binding Globulin (CBG) the strong exponential relationship between C assessed in the two media; and the improved

linear correlations when subjects known to have increased Cortisol Binding Protein. Thus, an advantage of measuring Sal-C rather than total serum cortisol is that it eliminates the need to account for within-subject changes or between-subject differences in CBG[36].

Plasma adrenocorticotrophic Hormone (ACTH) correlated significantly with the C concentrations determined 15 minutes later in serum and in saliva. Sal-C response is more pronounced than in serum and shows closer correlation to ACTH offer advantages over serum cortisol, suggesting Sal-C measurement may be used as an alternative parameter in dynamic endocrine test[37]. Regression found between serum and Sal-C concentrations permits the validation of saliva-sampling as a noninvasive technique for C level assessment in horses[38].

Serum C and Salivary Alpha amylase (SAA) levels are increased during pregnancy. During the luteal phase of the ovarian cycle, Sal-C levels increase, whereas serum C and SAA levels decline[39]. Sal-C is an acceptable surrogate for free serum cortisol when satisfactory salivary volumes are procured. Due to inadequate sample volumes, and contamination, it should not be generally adopted in the Intensive Care Unit. The analysis of both free serum cortisol via ultra filtration and Sal-C involved two steps: sample centrifugation followed by ELISA, suggesting consideration of widespread adoption of free serum cortisol in future investigations[40].

Sal-C concentration was found to be directly proportional to the serum unbound cortisol concentration both in normal men and women and in women with elevated CBG. The correlation was excellent in dynamic tests of adrenal function (dexamethasone suppression, ACTH stimulation), in normal and patients with adrenal insufficiency, in tests of circadian variation and randomly collected samples. Women in the third trimester of normal pregnancy exhibited elevated Sal-C throughout the day. The relationship between salivary and serum C concentration was markedly non-linear with a more rapid increase in salivary concentration once the serum CBG was saturated. The rate of equilibrium of cortisol between blood and saliva was very fast, being much less than 5 minutes. These data, combined with a simple, stress-free, non-invasive collection procedure, lead us to suggest that Sal-C is a more appropriate measure for the clinical assessment of adrenocortical function than is serum cortisol[41].

Salivary levels of Luteinizing hormone (LH), FT and DHEA-S correlate with their corresponding serum values, with a higher sensitivity of salivary more than serum approach[42]. Six to eight days after the LH surge salivary progesterone values of 300-800 pmol/L can be regarded as 'normal' and can therefore be used as a reference for the clinical assessment of infertile women[43]. There was a significant correlation between P and 17-OHP. When calculating ratios of P/Sal-Cand17-Hydroxyprogesterone (17-OHP) /Sal-C, linear regression yielded a much stronger correlation, although Sal-C did not show any correlation to P or 17-OHP[44].

The practical application of a sensitive saliva 17-OHP assay permitted detailed monitoring of patients receiving various glucocorticoid preparations through repeated frequent saliva sampling over the whole day. When the results of serial steroid measurements were analysed in relation to growth velocity in prepubertal patients, it was possible to device upper limits of 40 nmol/L, 0.8 nmol/L and 1,500 pmol/L for plasma 17-OHP, plasma testosterone and saliva 17-OHP concentrations, respectively, in well-controlled patients. Applying these guidelines from the early onset of treatment should ensure normal growth potential in treated congenital adrenal hyperplasia children, at least until puberty[45].

Salivary 8-hydroxy-2'-deoxyguanosine (Sal 8-OHdG) is a useful biomarker for predicting severe erectile dysfunction and hypogonadism in middle-aged men. Once-a-week treatment with sildenafil can have beneficial effects on men's health by decreasing oxidative stress and increasing testosterone levels[46].

Ovarian function is altered in a significant number of infertile women with endometriosis. However, these alterations are often subtle and only detected by detailed investigation[47]. There were no statistically significant correlations between Sal-C concentrations, FPI results, and age, number of poor responders, live birth rate, and clinical pregnancy rate (PR). Stress, as measured by Sal-C and the FPI questionnaire, does not negatively impact the effectiveness of ovarian hyper stimulation and is not associated with a reduced number of oocytes[48]. Since correct assessment of luteal function in basal conditions and during therapy requires multiple steroid measurements, and since saliva can be obtained by non-invasive techniques, salivary assays represent an attractive alternative to plasma for monitoring ovarian activity, also during specific treatment[49]. The mean concentration in the follicular phase, as determined, was 40.4 pmol/L (range 15.3-110.7 pmol/L). It increased in the peri-ovulatory period to a peak of 201.1 pmol/L (range 46.4-289.8 pmol/L) 6 days following follicular rupture[50].

Patients with prolonged unexplained infertility represent a heterogenous population with common luteal phase defects. The disturbance is effectively corrected with treatments stimulating gonadotropin secretion. These data provide more evidence for applicability of Sal-P measurements for diagnosis of corpus luteum function and highlights the difficulty of selecting representative reference populations in studies on female reproductive endocrinology[51]. Saliva steroid levels reflect the unbound unconjugated (free, biologically active) plasma hormone levels such as oestriol (E3), oestradiol (E2), oestrone (E1) and progesterone. The overall percentage increases in the median concentrations of E3, E2, E1 and progesterone were 718, 370, 80 and 214%, respectively, in the last 20 weeks and 149, 82, 24 and 41%, respectively, in the last 6 weeks of pregnancy[52]. Preterm labour without prior prolonged rupture of the membranes is, like term labour, preceded by an increase in the saliva oestriol to progesterone ratio. It may therefore be possible to use this ratio to predict preterm labour[53].

A statistically significant correlation between salivary and plasma levels of oestrone was shown in healthy men. This indicates the possibility of assaying saliva rather than plasma in endocrinological investigations[54]. The determination of steroids in saliva provides useful information in the clinical study of various endocrine functions, and Sal-P concentration has been widely used for assessing corpus luteum function during the spontaneous menstrual cycle and early pregnancy. There is a significant difference between fertile and non-fertile cycles with a higher saliva/plasma ratio in conception cycles[55]. There was only a weak relationship between the free hormone concentrations estimated in serum and the levels measured in saliva[56]. The levels of E2 in saliva followed the same pattern as in serum. The peak of E2 in saliva could be used to predict accurately the time of onset of the next menstrual period. The analysis of saliva could be useful in the investigation of women in whom serial vene punctures are not possible[57].

Chronic Migraine (CM) women showed significantly higher values than controls. Moreover, testosterone/cortisol ratios (anabolic/catabolic index of physical performance) were significantly lower in CM patients than in controls. CM appears not to be associated with an impairment of cortisol and DHEA-S circadian fluctuation; however, CM patient's present alterations in Hypothalamus Pituitary Axis (HPA) function that might contribute to metabolic and psychological alterations that have also been associated with CM[58]. Significant correlations were found between Sal-C, DHEA-S and α-amylase levels. The results showed that cortisol and DHEA-S concentrations were inversely correlated with α-amylase levels. Sal-C and DHEA-S concentrations reflect the activity of the HPA axis, whereas α-amylase activity is more closely related to sympathetic activity. Confirming that multiple saliva sampling (especially within 1 h after awakening) is necessary to reliably characterise biomarker activity when investigating neuroendocrine changes under various conditions[59].

DHEA-S results also suggest that DHEA-S levels change across the day and that future studies need to take this time of day difference into account when measuring DHEA-S[60]. For cortisol levels, both methods of saliva collection correlated highly with serum levels and with each other. For DHEA-S levels, only saliva samples collected using the unstimulated collection method correlated with plasma levels. DHEA-S collected using the salivette device did not correlate significantly with either plasma or the unstimulated saliva. It is crucial that future studies are aware of these issues and are cognizant of the effects of the method of collection when examining steroid levels in saliva[61]. DHEA-S can be measured accurately using passive drool or cotton Salivette collection methods. Results also suggest that DHEA-S levels change across the day and that future studies need to take this time of day difference into account when measuring DHEA-S[62]. The relation between the cortisol concentrations in serum and saliva was non-linear the satisfactory precision of the analysis and the simple non-invasive sampling procedure suggest that saliva may be used for cortisol measurements in situations where blood sampling is difficult to perform[63].

Salivary human Growth hormone (hGH) concentrations were 1000-fold lower than the respective values in serum, but a clear correlation was found between salivary and serum hGH levels[64]. Morning and evening salivary cortisol levels were correlated with sleep parameters in and insomnia in healthy controls. Sal-C at the time of awakening correlated negatively with the subjective estimation of sleep quality awakening cortisol was negatively correlated with the Pittsburgh Sleep Quality Index and with a questionnaire on sleep-related cognitions with the subscales rumination in bed and focusing on sleep-related thoughts[65]. A positive correlation was found between psychological factors and Sal-C levels in the oral lichen planus patients. The values of Pearson's correlation coefficient "r", between depression, anxiety, and stress with salivary cortisol were: +0.42, S; +0.27, NS; and +0.65, HS, respectively among the study group[66].

Although, the studies on correlation between saliva cortisol concentrations and free levels of this hormone in blood samples are lacking, Sal-C offer a novel approach in research of stress biomarkers with its ease of collection and potentially wide scope for application[67]. C and DHEA-S levels change with age and that the negative slope of DHEA-S was steeper than that of cortisol in saliva and serum. As the C and DHEA-S levels in saliva reflected those in serum, the measurement of steroid levels in saliva provide a useful and practical tool to evaluate adrenal functions, which are essential for clinical diagnosis[68]. Since C is also a mediator of stress-related negative effects on health and the DHEA-S/C ratio has been hypothesized as an index for the degree to which an individual is buffered against the negative effects of stress, these data might suggest potentially increased stress-related risks at early stages of male puberty[69].

2. Conclusion

This review article has highlighted the clinical usefulness of measuring reproductive hormones such as Testosterone, Cortisol, Progesterone, DHEA-S, SHBG, Oestradiol, Oestriol, and Oestrone in saliva. Many Studies have proved that measurements of all the above hormones reflect the free forms unlike the level usually measured in serum which is in bound form. Further, ease of sampling makes the use of saliva as a convenient non invasive procedure. Since many studies have established good correlations between salivary hormones to serum levels, hormones measured using saliva could be useful in the diagnosis of hypoganodism, testicular function, infertility, hirsutism, PCOS and stress related disorders. The contents of this paper will make awareness on the use of saliva in the near future for the diagnosis of all endocrine disorders. Further, the contents of this paper will help research field to undertake many more studies using saliva for clinical diagnosis of endocrine problems.

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