Evaluation of Thyroid Profile in Human Saliva with Special Reference to Ovulation

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Abstract: The precise thyroid profile like thyroid stimulating hormone (TSH), T₃, T₄ and luteinising hormone (LH) concentration was determined in saliva in order to establish the quantitative differences that might help in the assessment of ovulation by noninvasive method. The saliva from 20 different women volunteers during various reproductive phases (prepubertal, preovulatory, ovulatory, postovulatory phases and menopause) was collected and hormone concentrations were determined using radio immunoassay (RIA). Data of the present study indicated hormone like LH and T₄ was significantly augmented during the ovulatory phase as compared to other phases of the menstrual cycle. Hence, these salivary hormones like LH and T₄ could be used as biomarkers for detecting ovulation in human.

Key words: Saliva, luteinising hormone, thyroid hormone, menstrual cycle, noninvasive method

INTRODUCTION

Hormonal communication, which relies on the production and release of hormones from various glands and on the transport of these hormones through the bloodstream, is better suited for situations that require more widespread and longer lasting regulatory actions. Thus, the two communication systems, stimuli from the nervous system can influence the release of certain hormones, which complement each other. In addition, both systems interact: Generally speaking, hormones control the growth, development, and metabolism of the body, the electrolyte composition of body fluids, and reproduction.

Thyroid hormone in general serves to increase the metabolism of almost all body tissues. For example, thyroid hormone stimulates the production of certain proteins involved in heat generation in the body, a function that is essential for maintaining body temperature in cold climates. Moreover, thyroid hormone promotes several other metabolic processes involving carbohydrates, proteins, and lipids that help to generate the energy required for the body’s functions[1]. In addition to those metabolic effects, thyroid hormone plays an essential role in the development of the central nervous system during late fetal and early postnatal developmental stages. Furthermore, thyroid hormone exerts an effect similar to that of growth hormone (GH) on normal bone growth and maturation. Finally, thyroid hormone is required for the normal development of teeth, skin, and hair follicles as well as for the functioning of the nervous, cardiovascular, and gastrointestinal systems. T₄ constitutes approximately 90 percent of the hormone produced in the thyroid gland. However, T₃ is a much more active hormone, and most of the T₃ produced by the thyroid is converted into T₂ in the liver and kidneys.

During each menstrual cycle, the structure and possibly the function of genital organs and ovaries are modulated by cyclic hormonal changes of the demonstrated hypothalamic–pituitary–ovarian axis. Some of these changes can be assessed by basal body temperature (BBT), ferning examination, ultrasound, particularly transvaginal sonography alone or combined with the three-dimensional (3D) ultrasound and Doppler technique[5]. The day of ovulation is designated either as the day of maximum follicular growth, or as the day of follicle rupture[6]. Hormones used to measure in blood have been now estimated in saliva, though the quantities are comparatively less[6]. Hence, saliva is considered as the best non-invasive source for chemical and biochemical study[8]. Report shows that saliva is a very good source for both hormones and enzymes and their levels changed in accordance with the phases of menstrual cycle[9]. The numerous methods of hormonal assays described to predict ovulation[7–9] are all retrospective.

A noninvasive method for evaluation of luteal function is the need of the hour. A single assay of serum progesterone is not sufficient to characterize the duration or adequacy of luteal activity and cannot
detect the onset of progesterone secretion at the time of ovulation. Progesterone determined the concentration of pregnanediol, in early morning saliva specimen as a means of detecting the occurrence of ovulation \cite{10} and as a method for describing luteal function during normal menstrual cycle. The importance of predicting human ovulation for either optimizing or avoiding conception has been considered from an endocrine, morphological and clinical viewpoint. This study evaluates the reliability of a simple appreciation of salivary fern change at different levels as an ovulation indicator can be correlated with BBT and hormonal parameters such as luteinising hormone (LH) and thyroid profile like thyroid stimulating hormone (TSH), T₃ and T₄ in human saliva.

**MATERIAL AND METHODS**

**Subjects:** Consenting subjects were given a box containing 32 vials with 0.5 mg of NaN₃ dried in the bottom as a preservative and sugarless chewing gum for saliva collection at the beginning of each collection cycle. The subjects returned boxes with saliva samples to the clinic at the end of each monthly collection period. Collections were scheduled to be repeated every 6 months for three cycles.

Beginning on the first day of menstrual bleeding, subjects began saliva collection. In the morning prior to food intake or tooth brushing, subjects chewed one-half a stick of sugarless gum to increase salivation and deposited 7–10 ml of saliva into a vial, mixed the saliva with the preservative, and recorded the date of saliva collection on the vial and whether menstrual bleeding was present. This procedure was repeated daily for 30 days or until the beginning of the next menstrual period.

Saliva was stored in a light-tight box in the subject’s home without refrigeration and was brought to the Reproductive endocrinology lab, Department of Animal science, Bharathidasan University, Tiruchirappalli, at the end of the month. Progesterone concentrations in samples prepared in this way are completely stable for at least 2 months \cite{11}. The stability of thyroid profile and LH was tested again in this study by repeating assays of four samples that were stored at room temperature. No deterioration was observed in thyroid concentrations for four months. In nonmenstrual subjects the saliva samples were also tested the thyroid profile like TSH, T₃ and T₄ in human saliva.

**Sample and Assays:** Upon receipt, samples were stored at −20°C. For analysis the samples were thawed and centrifuged for 30 min at 5000 g to remove cellular debris. Batches of samples consisting of two month’s collections were prepared to include two quality control preparations for estimating assay precision. The quality control preparation was a pool of saliva that had been stripped of steroids by incubation with agarose-coated charcoal and to which 50 mIU/ml of LH and 25 mIU/ml of thyroid profiles had been added. LH and thyroid profiles were assayed by direct radio immunoassay as described in detail previously \cite{12,13}. Salivary LH assay \cite{14,15}; Salivary TSH \cite{16}; Salivary T₃ \cite{17}; Salivary T₄ \cite{18}. The cross reactivity of this antiserum were 0.26%, 1%, non-detectable and 0.001% respectively. The intra and inter assay coefficients of variation were 0.12-1.0 mIU/L for LH.

**Statistical Analysis:** Results were expressed as Mean ± SEM. Statistical analysis was performed by applying the statistical package for social sciences (SPSS/PC: SPSS-9, Chicago, USA). One-way analysis of variance (ANOVA) was applied to compare the mean values of different groups of infertility followed by multiple comparison post-Hoc Tukey HSD test. A “p” value of <0.001 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Results:** Our results indicate that simple appreciation of ovulatory saliva change is an accurate ovulation indicator, better than BBT and similar to the thyroid contents. Differences in evaluation times of the various ovulation indicators that were studied must be considered to interpret their relationship. A woman’s detection of saliva ferning requires observation during the course of a whole day. At the end of the day, the most fertile signs and symptoms pragmatic.

The mean level of the hormones in the follicular phase of ovulatory subjects was compared among the cycles within subjects. **Table 1** Shows the LH differences between preovulatory and postovulatory level was fairly significant (P<0.001). During the ovulatory phase, LH value showed the maximum range (90.35 ± 15.36 mIU/L), which is formed due to the fluctuation of preovulatory estrogen surge. The thyroid levels were consistently low during the major part of the preovulatory phase (Days 6-12 of the menstrual cycle, significantly varied among TSH as 6.90±0.522 mIU/ml; T₃ concentration was 8.89±1.27 mIU/ml; T₄ concentration was 11.96±1.12 mIU/ml) and increased beginning with the periovulatory phase up to 20 pg/ml (approximately). A high plateau reached between preovulatory and postovulatory (4.27±0.37 pmol/L) phases, then values decreased constantly (P<0.001) in other periods (Graph 1). In prepubertal, the level of TSH showed decrease in concentration (2.82±0.55 pmol/L). During the comparison with other Thyroid levels T₃ shows high concentration in prepubertal cases (5.48±1.66 pmol/L). During menopause the concentration of thyroid metabolites showed less
**Tabl e 1:** Hormones profile in human saliva (Mean ± SEM) during normal menstrual cycle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prepubertal (6-9 years)</th>
<th>Preovulatory phase (6-12 days)</th>
<th>Ovulatory phase (13-14 days)</th>
<th>Postovulatory phase (16-26 days)</th>
<th>Menopause (Above 45 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>5.69 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.56 ± 3.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.35 ± 15.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.36 ± 1.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.54 ± 1.05&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSH</td>
<td>2.82±0.55</td>
<td>4.27±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.90±0.52</td>
<td>4.25±0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.47±0.48</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.62±0.63</td>
<td>5.59±0.75</td>
<td>8.89±1.28</td>
<td>4.56±0.70</td>
<td>4.43±0.83&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5.48±0.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.02±0.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.96±1.12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.93±0.58&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.17±0.77&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note: The value are expressed as mean ± SEM. Note twenty women used in all three phases with triplicate analysis and compared with prepubertal, menopause periods. The significant difference between all phases prove in one way – ANOVA with Posthoc Tukey HSD test (p£ 0.001)

<sup>a</sup> Prepubertal vs preovulatory phase  
<sup>b</sup> Preovulatory phase vs ovulatory phase  
<sup>c</sup> Ovulatory phase vs postovulatory phase  
<sup>d</sup> Postovulatory phase vs menopause  
<sup>e</sup> Menopause vs prepubertal

**Graph 1:** Hormone profile in human saliva during normal menstrual cycle

Concentration compared to other periods. In non-menstrual subjects the salivary hormones like LH, TSH, T<sub>3</sub> and T<sub>4</sub> show the presence of sufficient intensity, which might be due to the formation of hypothyroidism.

**Discussion:** The present study revealed that the nature of saliva considerably varied depending upon the reproductive status of women. Salivary steroid concentrations are noted throughout to reflect the free-steroid concentration in plasma<sup>[19]</sup>. In plasma of normal subjects the ratio of total T<sub>3</sub> to free T<sub>3</sub> is approximately 20 pmol/L during ovulatory phase with a free T<sub>4</sub> concentration of about 11.96 pmol/L. It was not possible to assess the T<sub>4</sub> concentration in saliva accurately because of limited assay sensitivity. However, our results suggest that, although most individuals have saliva T<sub>4</sub> concentrations less than 20 pmol/L, some samples may exceed this value. The protein binding of T<sub>4</sub> in whole saliva is probably due to the trace contamination of the saliva with plasma. It was found that a 5000-fold dilution of plasma in assay buffer would bind a similar amount of T<sub>3</sub> tracer. Because of the very large total-to-free ratio for T<sub>4</sub> in plasma, contamination of saliva with plasma or gingival fluid it is likely to cause a large variability in the concentration of T<sub>4</sub> in saliva. Correspondingly, it is found that the T<sub>4</sub> binding capacity of saliva varied markedly between individuals and within individuals on consecutive days. Accordingly, measurements of the T<sub>4</sub> concentrations in whole saliva are unlikely to give an accurate index of the plasma concentrations of free T<sub>4</sub>.

Apart from these vascular effects, estrogen has also been suggested to have direct effects on thyroid function<sup>[20]</sup>, and it showed that exogenous estrogen in oral contraceptives increases TSH response to thyrotropin-releasing hormone (TRH) by increasing the number of TRH receptors in anterior pituitary<sup>[20,21]</sup>. The changing pattern of thyroid blood flow seen in the present investigation was consistent with the changes of the serum levels of TSH and metabolites like T<sub>1</sub>, T<sub>4</sub> and thyroglobulin (Tg), and thyroid volume during normal menstrual cycle<sup>[22]</sup>. In the normal menstrual cycle, the slight decrease in the level of estrogen at ovulation leads to a decrease of TSH level from the follicular phase to the ovulatory phase, and this leads to a decrease of Tg level, because Tg production is stimulated by TSH. The decreased Tg level stimulates the feedback mechanisms and increases thyroid metabolism to regulate the serum levels of thyroid hormones.
Increased thyroid metabolism increases thyroid volume and perfusion in the thyroid vascular bed in this period. From the ovulatory to luteal phase, the increased serum level of estrogen increases the levels of TSH and Tg. The rate of thyroid metabolism is then slowed down so that the production of thyroid hormones is kept in the normal range. The thyroid metabolic rate in the luteal phase was still higher than that in the ovulatory phase, and this may be due to a sustained higher level of TSH responding to the higher level of estrogen. Thyroid volume is thus increased, but only to a small degree. This may explain why the slope for the increase of thyroid volume from Day 16 to Day 23 was much flatter than that from Day 9 to Day 16\(^{[21]}\). A decrease of thyroid perfusion from the ovulatory to the luteal phases may be indicated by the slight increase of the mean pulsatility index, though this may also be partly due to the increased level of progesterone, which has a vasoconstrictor effect on the blood vessels \(^{[23]}\). In this study, the effect of estrogen on thyroid metabolism is suggested, although the details of how female sex hormones affect thyroid metabolism are still unknown. In general, if there is a stimulatory effect of estrogen on thyroid function, thyroid perfusion in women is expected quantitatively to be higher than that in men. The menstrual pattern is influenced by thyroid hormones directly through impact on the ovariies and indirectly through impact on SHBG, PRL and GnRH secretion and coagulation factors. Treating thyroid dysfunction can reverse menstrual abnormalities and thus improve fertility. In infertile women, the prevalence of autoimmune thyroid disease (AITD) is significantly higher compared to parous age-matched women. This is especially the case in women with endometriosis and polycystic ovarian syndrome \(^{[24]}\).

The lipid-soluble unconjugated steroids (such as cortisol, estriol, testosterone, progesterone, etc.) enter saliva predominantly via the intracellular route; their salivary concentrations are not dependent on saliva flow rate, and their salivary concentrations closely approximate their unbound concentration in plasma. Accordingly, the salivary concentration of these hormones may provide a useful clinical index of their unbound concentrations in plasma. Cyclic changes in the concentration of this LH like material in saliva fluctuate to prove a biologic role during the normal menstrual cycle. Since LH like material is much higher than in serum, it is possible to speculate the possible existence of receptor proteins in saliva or other concentrating secretory mechanism. The RIA provides significant results that the thyroid hormone metabolites like T4 expressed predominantly only during the mid cycle.

Our results seem to point in the same direction but were not statistically significant. In addition, BBT is routinely used to detect ovulation in infertility investigation and to monitor the effect of ovulation inducing agents. We have found that BBT is less accurate. This study proved that simple appreciation of fern pattern of saliva showed the change is an accurate ovulation indicator. Hormonal ovulation detection that measures salivary LH and thyroid profiles does not seem to be better. Other clinical or laboratory methods such as evaluation of endometrial biopsy or assay of pregnandiol might help in establishing the presence or absence of ovulation. All these findings can have clinical application in family planning and infertility.

**REFERENCE**


