Salivary and serum cortisol levels, salivary alpha-amylase and unstimulated whole saliva flow rate in pregnant and non-pregnant women

Níveis de cortisol salivar e sérico, alfa-amilase e fluxo de saliva total não estimulada em gestantes e não gestantes

Abstract

PURPOSE: To compare salivary and serum cortisol levels, salivary alpha-amylase (sAA), and unstimulated whole saliva (UWS) flow rate in pregnant and non-pregnant women. METHOD: A longitudinal study was conducted at a health promotion center of a university hospital. Nine pregnant and 12 non-pregnant women participated in the study. Serum and UWS were collected and analyzed every trimester and twice a month during the menstrual cycle. The salivary and serum cortisol levels were determined by chemiluminescence assay and the sAA was processed in an automated biochemistry analyzer. RESULTS: Significant differences between the pregnant and non-pregnant groups were found in median [interquartile range] levels of serum cortisol (23.8 µL/dL [19.4–29.4] versus 12.3 [9.6–16.8], p<0.001) and sAA (56.7 U/L [30.9–82.2] versus 31.8 [18.1–53.2], p<0.001). Differences in salivary and serum cortisol (µL/dL) and sAA levels in the follicular versus luteal phase were observed (p<0.001). Median UWS flow rates were similar in pregnant (0.26 [0.15–0.30] mL/min) and non-pregnant subjects (0.23 [0.20–0.32] mL/min). Significant correlations were found between salivary and serum cortisol (p=0.02) and between salivary cortisol and sAA (p=0.01). CONCLUSIONS: Serum cortisol and sAA levels are increased during pregnancy. During the luteal phase of the ovarian cycle, salivary cortisol levels increase, whereas serum cortisol and sAA levels decline.

Resumo

OBJETIVO: Comparar os níveis de cortisol sérico e salivar, alfa-amilase salivar (sAA) e fluxo de saliva não estimulada (UWS) em gestantes e não gestantes. MÉTODOS: Tratase de um estudo longitudinal realizado no centro de promoção da saúde de um hospital universitário. Nove gestantes e 12 não gestantes participaram do estudo. Foram coletados e analisados soro e UWS nos três trimestres gestacionais e duas vezes por mês durante o ciclo menstrual. A análise do cortisol salivar e sérico foi realizada com o uso de quimiluminescência e a atividade da sAA foi determinada por meio de analisador automático para bioquímica. RESULTADOS: Foi verificado que a mediana [intervalo interquartil] dos níveis de cortisol sérico no grupo de gestantes foi maior que 23,8 µL/dL [19.4–29.4] quando comparado ao grupo de não gestantes, que teve média de 12,3 [9.6–16.8; p<0.001]. Os níveis de sAA seguiram o mesmo padrão, com médias de 56,7 U/L [30.9–82.2] e 31,8 [18.1–53.2; p<0.001], respectivamente. Foram observadas diferenças dos níveis de cortisol sérico e salivar (µL/dL) e de sAA entre a fase folicular versus a fase lútea (p<0,001). As medianas dos fluxos salivares [UWS] foram semelhantes em gestantes [0.26 [0.15–0.30] mL/min] e não gestantes [0.23 [0.20–0.32] mL/min]. Foram encontradas correlações significativas entre o cortisol salivar e o sérico (p=0.02) e entre o cortisol salivar e a sAA (p=0.01). CONCLUSÕES: Os níveis de cortisol sérico de sAA durante a gestação elevam-se. Na fase lútea do ciclo ovariano, os níveis de cortisol salivar aumentam ao passo que os níveis de cortisol sérico e sAA diminuem.
Salivary and Serum Cortisol Levels, Salivary Alpha-Amylase and Unstimulated Whole Saliva Flow Rate in Pregnant and Non-Pregnant Women

Introduction

Laboratory analysis of saliva has become an important technique for the assessment of physiological and pathological conditions, mostly due to the origin, composition, and functions of saliva, as well as its interactions with other body systems and structures. Other favorable aspects of saliva testing include painless sampling, ease of storage, and low cost of analysis as compared with blood. These factors have driven extensive research into this testing modality1-3, including validation studies of quantitation of a variety of organic and inorganic compounds in saliva4.

Cortisol is a hormone secreted by the adrenal glands that can be detected in urine, serum, and saliva. Measurement of cortisol levels in saliva is gaining increasingly widespread acceptance as a diagnostic method because they correspond only to the unbound, bioactive fraction of cortisol, whereas most serum cortisol is bound to proteins such as corticosteroid-binding globulin (CBG)5,6. Salivary cortisol testing has been used to assess hypothalamic-pituitary-adrenal (HPA) axis function under various cognitive conditions and in the presence of stress and anxiety6,7. During pregnancy, baseline salivary cortisol concentrations exhibit a constant increase starting around gestational week 25; by term, levels are over twice as high as those detected in non-pregnant women8. Within one week after delivery, salivary cortisol levels return to baseline8. The physiology of cortisol can be assessed under baseline conditions and in response to specific stressors9,10. Measurement of changes in baseline cortisol levels and in cortisol reactivity to stress during pregnancy is important, as high concentrations of cortisol affect fetal development7,10,11 and may lead to low birth weight12.

The enzyme salivary alpha-amylase (sAA) is one of the key protein constituents of saliva and accounts for 10–20% of all proteins produced by the parotid gland13. Its function includes, but is not restricted to, initiation of digestion in the oral cavity. It also plays a major role in modulation of bacterial adhesion and growth on intraoral surfaces14. Recent studies have highlighted the utility of sAA as a marker of physical, psychological, or psychosocial stress induced by activation of the autonomic nervous system, which controls the salivary glands13,15-17. Furthermore, increased levels of sAA have been shown to reduce the likelihood of conception during the fertile window in women18.

Pregnancy-related changes in sAA secretion have rarely been described in the literature. Studies suggest that salivary flow and sAA levels remain unchanged during gestation19,20. However, a research has also shown that pregnant women exposed to stressor agents exhibit increased sAA concentrations17; conversely, other study have demonstrated less marked changes in sAA levels in response to stress in pregnant versus non-pregnant subjects3.

Salivary cortisol and sAA have been used in medical and psychological research as physiological and psychological markers of psychosocial stress13,21-23. However, data on baseline sAA and cortisol levels during the menstrual cycle in humans are scarce, conflicting, and inconclusive in relation to changes during pregnancy5,19,20,24.

The aim of the present study was to measure serum and salivary cortisol levels, sAA and unstimulated whole saliva (UWS) flow rate in pregnant and non-pregnant women and compare these levels during each trimester of pregnancy (in the pregnant group) and during the follicular and luteal phases of the ovarian cycle (in the non-pregnant group). A secondary objective was to ascertain whether correlations exist between these variables.

Methods

Pregnant and non-pregnant women seen at Hospital Universitário da Universidade de Brasília, in Brasília, Brazil, were invited to take part in this longitudinal study. The criteria for inclusion common to both groups were good overall health, age > 18 years, no history of miscarriage during the last 2 years, no current systemic pharmacotherapy, and no smoking. Pregnant subjects were required to be in the first trimester, and non-pregnant participants were required to refrain from hormonal contraceptive use. All participants underwent an intraoral examination, interview and history-taking, and blood and UWS collection in each trimester of pregnancy (for pregnant participants) and during the follicular and luteal phases of the menstrual cycle (for non-pregnant participants). The study was conducted according to the Declaration of Helsinki and all participants provided written informed consent approval by the Universidade de Brasília School of Medicine Research Ethics Committee (#040/07).

Case series and sample collection

In the pregnant group, first-trimester samples were collected between gestational weeks 11 and 16; second-trimester samples, between gestational weeks 18 and 22; and third-trimester samples, between weeks 32 and 36. All non-pregnant participants had regular cycles, and menstrual cycle phases were estimated on the basis of information provided during the interview. The self-reported date of onset of menses was used to calculate the follicular phase (6 to 8 days later) and luteal phase (23 to 25 days later).

Participants were instructed to arrive after at least 8 hours of fasting and to have completed their routine oral hygiene within 2 hours before sample collection. Blood samples were collected first, between 7:00 and 8:00 a.m., followed by UWS. Patients were seated on a
dental chair for 2 minutes to relax and then instructed to spit, so as to discard any detritus-containing saliva present in the oral cavity. This was defined as time point zero for collection. Participants remained seated, with eyes open and the neck and head flexed forward to facilitate “passive” flow of saliva, and were instructed to refrain from moving the tongue, cheeks, or lips. UWS was collected into a 50-mL Falcon® polypropylene tube. Overall collection time was 6 minutes. Samples exhibiting reddish discoloration (suggesting presence of blood) or cloudiness or turbidity (suggesting excessive epithelial cell shedding) were discarded to prevent excessive variation in cortisol and sAA levels. The collected samples were immediately sent for analysis. The UWS flow rate was expressed as volume of saliva/unit of time (mL/min).

**Laboratory analysis of cortisol and sAA levels**

For measurement of cortisol levels, saliva samples were centrifuged and the supernatant set aside for analysis. Cortisol levels in serum and saliva (µg/dL) were determined by chemiluminescence assay (Immulite 2000®, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), using reagents and calibration materials provided by the manufacturer. A 10 µl aliquot of serum/saliva was used and the calibration curve ranged from 1–50 µg/dL for cortisol. For measurement of sAA (U/L), saliva samples were diluted in distilled water to a concentration of 1:100 (1%) and processed in an Architect c8000® automated chemistry analyzer (Abbott Clinical Chemistry, Wiesbaden, Germany), using reagents and calibration materials provided by the manufacturer.

**Statistical methods**

The sample size was calculated for a two-sided test to have 80% power to detect a clinically significant mean (standard deviation) difference of 4.4 (3.5) µg/dL in salivary cortisol between groups, based on previous study. Alpha was set at 0.05. The total number of subjects to be recruited was 20. Nevertheless, it was assumed an attrition rate of 30%, which is to be expected in a long-term study once pregnant women are susceptible to intercurrent conditions. Furthermore, it was also considered that non-pregnant subjects were asked to attend study visits repeatedly without any health reason, nor monetary incentive to do so. Hence, it was enrolled 26 participants.

Data were analyzed in the Statistical Package for the Social Sciences (SPSS® 20.0 for Windows, SPSS Inc., IBM Group, Chicago, USA). All tests were two-sided and the significance level was set at p<0.05. Initially, the Mann-Whitney U test was used to assess between-group differences (pregnant versus non-pregnant). Afterwards, within-group differences were assessed. Serum cortisol, sAA and UWS flow rate were compared among pregnancy trimesters by means of analysis of variance (ANOVA), while the Kruskal-Wallis test was used for comparison of salivary cortisol levels. Within-group differences between the follicular and luteal phases of the ovarian cycle in non-pregnant subjects were assessed with the Wilcoxon test (salivary cortisol, sAA, and UWS flow rate) or a dependent t-test (serum cortisol). Spearman correlation coefficients were calculated for sAA, UWS flow rate, and serum and salivary cortisol.

**Results**

A total of 13 pregnant (primigravida) and 13 non-pregnant women were enrolled. Three miscarriages and one dropout occurred in the pregnant group, and one control was lost to follow-up. Thus, 9 primigravidas with median age (interquartile range) of 28 years (25–31), and 12 non-pregnant women aged 29 (27–32) took part in the study. A total of 27 and 24 samples were collected for each variable per group, respectively. There were no significant between-group differences in salivary cortisol levels. However, significant within-group differences in median (interquartile range) levels were found among the non-pregnant subjects, with values of 1.0 (1.0–1.05) µg/dL in the follicular phase versus 1.1 (1.0–1.48) µg/dL in the luteal phase (p<0.001) (Figure 1).

Median serum cortisol levels were significantly different in the pregnant and non-pregnant groups (23.8 [19.4–29.4] versus 12.3 [9.6–16.8] µg/dL, and also between follicular versus luteal phase (12.7 [10.2–18.7] versus 12.2 [9.1–15.1] µg/dL (p<0.001) (Figure 2).
There were no differences in median UWS flow rate values between pregnant and non-pregnant subjects (0.26 [0.15–0.30] versus 0.23 [0.20–0.32] mL/min), and no within-group differences among trimesters of pregnancy or between the follicular and luteal phases of the ovarian cycle (data not shown).

Levels of sAA were significantly different between the pregnant and non-pregnant groups (56.7 [30.9–82.2] versus 31.8 [18.1–53.2] U/L, p<0.001) and between the follicular and luteal phases in the non-pregnant group (p<0.001) (Figure 3).

Significant Spearman correlations were found between salivary and serum cortisol levels (p=0.02) and between salivary cortisol and sAA (p=0.01) (Table 1).

**Table 1.** Spearman correlation coefficients

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<th>UWS flow rate</th>
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</table>

*Correlation is significant at the 0.05 level (2-tailed).

UWS: unstimulated whole saliva.

**Discussion**

Measurement of salivary cortisol levels has been widely used as an alternative to quantitation of this hormone in plasma or serum. Saliva samples are readily obtained and can be collected several times a day, allowing dynamic assessment of free cortisol secretion. Circulating unbound cortisol is quickly transported to saliva by passive diffusion, to the extent that some studies report strong correlations between salivary cortisol levels and free (unbound) cortisol concentrations in plasma and serum.

However, if salivary cortisol levels are to be used for diagnostic purposes in clinical practice, analytical methods must be standardized and cutoff values defined on the basis of normal population-wide control samples to serve as a reference range for testing. Research and retail laboratories should validate their salivary cortisol assays before making them available to clinicians.

The interrelatedness between serum and salivary cortisol levels in non-pregnant women and men appears to be the same. However, women in the third trimester of pregnancy and those on oral contraceptives exhibit markedly increased serum cortisol levels, but near-normal salivary cortisol.

The linear correlation between serum free cortisol and salivary cortisol is usually very strong, independent of changes in CBG concentrations, and similar across all groups: men, pregnant women, non-pregnant women, and oral contraceptive users.

Conversely, some authors believe these parameters should be interpreted cautiously, as salivary cortisol concentrations do not correlate linearly with serum levels in some cases. This nonlinearity in association between total salivary and serum cortisol may be attributable to the presence of CBG in plasma. CBG concentrations may be increased during oral contraceptive use and in certain physiological conditions, such as pregnancy.
Although measurement of stimulated saliva is useful for research purposes, it is important to consider the potential differences between salivary and serum cortisol levels when interpreting results. Salivary cortisol levels may be influenced by factors such as salivary flow rate, which can vary due to various physiological and psychological factors. In the present study, salivary cortisol levels were measured in pregnant and non-pregnant women, and no significant differences were found. However, it is important to note that this study was performed in a small sample size, which may limit the generalizability of the findings to other populations.

In summary, while salivary cortisol levels may not always accurately reflect serum levels, they can still be a useful biomarker for assessing stress-related changes in the human body. Further research is needed to better understand the potential differences between salivary and serum cortisol levels and their implications for clinical practice.
diagnostics still require further research for standardization of analytical methods, validation of results, and definition of analyte reference ranges in a series of populations before they can be made available to clinical practice.

In conclusion, serum cortisol and sAA levels are higher in pregnant than in non-pregnant women. During the ovarian cycle, salivary cortisol levels increase in the luteal phase, whereas serum cortisol and sAA levels decline.

References


