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Day-to-day variation of late-night salivary cortisol in healthy voluntaries

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ABSTRACT

Objectives: The study examines the components of biological variation of nocturnal salivary cortisol in healthy subjects.

Design and methods: Eight repetitive measurements were performed in seven subjects during a 25-day time period (study A), and then, for comparison, two salivary specimens were taken during two consecutive days from 20 subjects (study B). Salivary cortisol was measured with the Salimetrics HS-Cortisol assay.

Results: Mean salivary cortisol (1.27 nmol/L), analytical variation (CV*a* = 15.4%), within-subject variation (CV*i* = 34.1%), between-subject variation (CV*g* = 35.3%), index of individuality (II = 1.06) and reference change value (RCV = 104%) were obtained for study A. Similar results were obtained from the set of samples of study B.

Conclusion: The study results show a medium degree of individuality for salivary cortisol. Both conventional reference values and comparison of serial results may be equally used for clinical interpretation. A change greater of 104% between two successive measurements should be considered significant.

Introduction

Salivary cortisol is an excellent indicator of plasma-free cortisol [1,2] increasingly used to assess hypothalamic–pituitary–adrenal axis secretory activity and rhythm [3–6]. At present it is widely accepted that late-night salivary cortisol measurement is a simple and reliable way to screen patients for Cushing's syndrome [7–11]. In fact, the Clinical Guideline Committee of the Endocrine Society recommends the use of nocturnal salivary cortisol as a first step procedure in the diagnosis of Cushing's syndrome [12]. Two salivary cortisol measurements per subject of different salivary collections are recommended [12]. However, magnitude of day-to-day intra-individual and between-subject variations of late-night salivary cortisol concentrations in an outpatient setting is unknown. Data on the biological variations of analyte concentrations are necessary for judging the usefulness of conventional population-based reference intervals, correctly interpreting serial laboratory results from a single patient in clinical practice and objectively determining the analytical goals required to facilitate optimal patient care [13–15].

The aim of the present study was to determine the within- and between-subject biological variation of late-night salivary cortisol by using individual means obtained from day-to-day data in healthy subjects.

Materials and methods

Subjects and samples

Seven healthy volunteers (four females/three males; age 26–65 years) with no clinical features of Cushing's syndrome were requested to enroll in a serial testing study (study A). A sample of saliva collected between 23:30 h and 0:00 h should be provided two times per week during four consecutive weeks. In a second study (study B), 20 additional healthy volunteers (13 females/7 males; age 25–65 years) with no clinical features of Cushing's syndrome were requested to provide two successive late-night saliva samples (two consecutive evenings), an approach commonly used in clinical practice for screening of Cushing's disease. Volunteers were provided with a sheet with collection directions and cotton swab devices (Salivette, Sarstedt, Nümbrecht, Germany) for saliva collection. The participants were directed to collect saliva at least 2 h after eating, smoking or brushing their teeth. Samples were stored at 4 °C overnight and frozen next morning at −20 °C upon arrival at laboratory. During the period of study any change in lifestyle habit (smoking, drugs, diet, stress) was kept to minimum. Studies were conducted in accordance with the guidelines of the declaration of Helsinki and informed consent was obtained from all participants.

Salivary cortisol measurements

At the end of the collection period, all frozen samples were thawed, mixed, centrifuged at 3000 g for 10 min at room temperature and analyzed in duplicate with a competitive immunoassay [16]

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specifically validated for the quantitative measurement of salivary cortisol (Salimetrics LLC, State College, Pennsylvania). The analytical sensitivity was 0.08 nmol/L and the calibration range was 0.33–82.8 nmol/L. Inter-assay coefficient of variation was 18% (2.8 nmol/L) and 11% (28 nmol/L). The analytical sensitivity was determined by the manufacturer by interpolating the mean minus two standard deviations for 10 sets of duplicates at 0 nmol/L standard. Determination of inter-assay coefficient of variation was performed in our laboratory by analyzing two levels of internal quality control material in 15 runs.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc.) and Excel 2008 (Microsoft Corp., USA). Investigation for outlier results for each individual's set of samples was performed by comparing each subject standard deviation to the mean of all standard deviations ± 3 SD. Cochran's variance test was used to investigate if any participant was an outlier from the group [13,17]. Based on these tests, one sample from a male subject was excluded. Then, different sets of data were taken to different calculations that are used in the clinical laboratory to estimate the biological variation and to facilitate the interpretation of the results. This included the calculation of the analytical coefficient of variation for cortisol in saliva (CVa), the within-subject coefficient of biological variation (CVi), the between-subject coefficient of variation (CVg), the index of individuality (II) and the reference change value (bilateral, RCV 95%) according to the approach of Fraser and Harris [13,14]. Analytical variance (SDa) was calculated from the differences between the duplicates according to the formula: $Sa^2 = \Sigma d^2/2N$, where d is the difference between duplicates and N is the number of duplicates. One-way analysis of variance was used to distinguish the total variance into between-subject variance (S^2g) and total within-subject variance ($S^2i + a$). Biological within-subject (intra-individual) variation (S^2i) was estimated from the total within-subject variance ($S^2i + a$) minus analytical variance, according to the formula: $S^2i = S^2i + a - S^2a$. The analytical, within- and between-subject biological variations were expressed as coefficients of variation (CVa [%], CVi [%] and CVg [%], respectively). The reference change value (RCV) or critical difference is the minimal significant difference ($p<0.05$) between two consecutive measurements in the same subject. It was calculated according to the following formula: $RCV(\%) = 2.77 \times (CV^2a + CV^2i)^{1/2}$. The index of individuality (II), $CVi + a/CVg$, describes the relationship between within-subject and between-subject variation and it was used to evaluate the usefulness of population-based reference values [15].

We also calculated the desirable quality specifications for imprecision (I), bias (B), and total error (TE), which were calculated using the formulas [18]: $I<0.5CVi$; $B<0.25(CV^2i + CV^2g)^{1/2}$ and $TE<1.65 \times I + B$ ($\alpha<0.05$).

Results

Salivary cortisol means and absolute ranges (minimum and maximum values) of the seven subjects that provided eight salivary samples (study A) are shown in Fig. 1. The median age (range) of subjects was 29 years (26–65). Mean salivary cortisol concentrations ranged from 0.69 to 2.01 nmol/L for subjects, with an overall mean of 1.27 nmol/L. There was no significant differences between mean salivary cortisol for women (1.21 ± 0.28 nmol/L) compared with men (1.46 ± 0.30 nmol/L).

Salivary cortisol means and absolute ranges (minimum and maximum values) of the 20 subjects that provided two consecutive salivary samples (study B) are shown in Fig. 2. The median age (range) of subjects was 31 years (25–65 years). Mean salivary cortisol concentrations ranged from 0.36 to 3.42 nmol/L for subjects, with an overall mean of 1.35 nmol/L. There was no significant differences between mean salivary cortisol for women (1.44 ± 0.39 nmol/L) compared with men (1.32 ± 0.14 nmol/L).

Fig. 1. Range of salivary cortisol concentrations in seven healthy subjects, measured by serial analysis with salivary collection between 23:30 and 00:00 over a 4-week period. $n = 8$ values for each subject. The empty boxes correspond to female individuals ($n = 4$; subjects 1, 4, 6 and 7). The filled boxes correspond to male individuals ($n = 3$; subjects 2, 3, 5) (Study A).

The means and the components of biological variation of salivary cortisol found in studies A and B, expressed in terms of CV, are shown in Table 1. Also shown in Table 1 is the index of individuality (II) and the reference change value (RCV). Table 2 displays the analytical goals for salivary cortisol based on the components of the biological variation obtained from studies A and B.

Discussion

Biochemical screening studies for Cushing's syndrome have traditionally included low-dose dexamethasone suppression testing, 24-h urine free cortisol measurement and evaluation of diurnal rhythmicity [8]. These studies are often cumbersome and may require hospitalization. At present, late-night salivary cortisol measurement is also recommended as a first step procedure in the diagnosis of Cushing's syndrome [12]. It is a much more practical way of screening for Cushing's syndrome since it can be easily performed on an outpatient basis without disrupting a normal routine. In addition, the

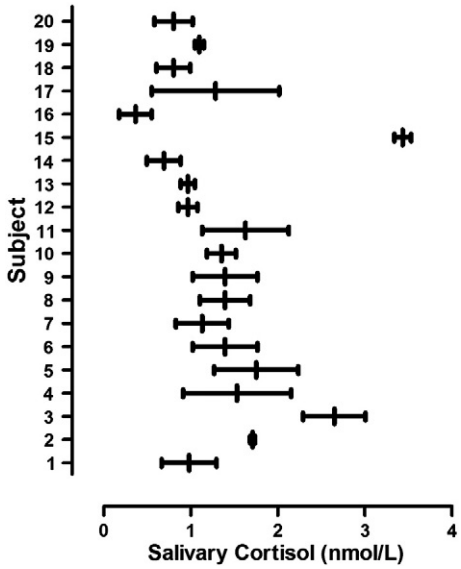

Fig. 2. Range of salivary cortisol concentrations in 20 healthy subjects measured in two consecutive salivary collections obtained between 23:30 and 00:00. Female subjects ($n = 13$) correspond to subjects 3, 4, 6–13, 17, 18, 20. Male subjects ($n = 7$) correspond to subjects 1, 2, 5, 14–16, 19 (Study B).