Salivary cortisol as an alternative for serum cortisol in the low-dose adrenocorticotropic hormone stimulation test?

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ABSTRACT. Background: Salivary cortisol is unaffected by cortisol binding globulin and reflects free serum cortisol as compared to total serum cortisol. Aim: The aim of the present study was to compare the salivary cortisol response with the serum cortisol response in a low-dose (1-μg) ACTH test in a clinical setting and to determine the optimal cut-off value of salivary cortisol as an alternative to serum cortisol. Material/subjects and methods: We measured serum and salivary cortisol responses to iv administration of 1-μg ACTH in 51 patients (17 males) referred to the Department of Clinical Chemistry for ACTH-testing. Serum cortisol was assessed before, 20, and 30 min after ACTH-administration, and salivary cortisol was assessed before and 30 min after ACTH administration. Results: Mean cortisol at baseline, 20, and 30 min were 0.44 μmol/l (SD: 0.22), 0.64 μmol/l (SD: 0.24), and 0.70 μmol/l (SD: 0.25), respectively. Median basal salivary cortisol was 8.4 nmol/l [interquartile range (IQR): 3.8-14.2]. Salivary cortisol at 30 min equaled 35.9 nmol/l (IQR: 21.1-46.2). Basal salivary cortisol was significantly correlated with salivary cortisol at 30 min (r=0.53; p<0.001). Salivary cortisol at 30 min of 23.5 nmol/l had a sensitivity and specificity of 78.1% and 70.0%, respectively as compared to the serum cortisol cut-off values of >0.50 μmol/l. Conclusions: The salivary low-dose ACTH-test yields more dynamic responses than serum cortisol. However, the sensitivity and specificity of salivary cortisol are too low to be adequate as an alternative to the serum cortisol measurements. In women on estrogen therapy, however, the use of salivary cortisol might be superior to serum cortisol.

INTRODUCTION

The low-dose (1 μg) ACTH stimulation test is used for the detection of primary or prolonged secondary adrenocortical insufficiency, correlates well with the insulin-induced hypoglycemia test (1-3) and is a sensitive test for the assessment of adrenal function (1). Indeed, a recent meta-analysis showed that the low-dose ACTH test had the highest sensitivity and specificity as compared to the 250-μg ACTH in the diagnosis of adrenal insufficiency (4). In most laboratory assays, changes in total serum cortisol i.e. the sum of the biologically active cortisol and protein-bound cortisol are determined after an ACTH test. However, cortisol binding globulin (CBG) may significantly influence total serum cortisol (5), for instance estrogens may increase CBG and therefore increase total serum cortisol (6). In saliva, cortisol is present in the unbound form and may very well reflect the free cortisol fraction in serum (7). In line with this conjecture, two studies found higher basal and stimulated serum cortisol in women who used oral contraceptives as compared to women who did not use oral contraceptives, whereas salivary cortisol was similar in both groups (8, 9). The use of oral contraceptives may thus hamper the interpretation of changes in total serum cortisol (10). Moreover, salivary cortisol after a low-dose ACTH test has shown a higher stimulatory response and a lower variability as compared to serum cortisol (9). In addition, the sampling of saliva is easy and may provide a stress-free and non-invasive alternative to serum cortisol in clinical practice and in research settings (11). A limited number of studies reported on the comparison of salivary cortisol with serum cortisol after a low-dose ACTH test (8, 9, 12). However, these studies were performed in selected populations in a research setting and did not report sensitivity and specificity of the measurement of salivary cortisol as compared to serum cortisol. Therefore, in the present study we compared the salivary cortisol response to the serum cortisol response in a low-dose (1 μg) ACTH test in a clinical setting and determined the optimal cut-off value of salivary cortisol as an alternative to serum cortisol.

MATERIALS AND METHODS

Patients

In the present study, 51 consecutive patients (17 males; age: 18-90 yr) were included, who were referred to the outpatient facility of the Department of Clinical Chemistry at the Isala Clinics in Zwolle, the Netherlands for a low-dose (1-μg) ACTH test by one of the endocrinologists from the Department of Internal Medicine. The indications for referral to the laboratory were as follows: chronic fatigue (no.=37), follow-up after pituitary surgery (no.=4), hypotension (no.=4), Addison’s disease (no.=2), pituitary cyst (no.=1), pituitary function after septic shock (no.=1), adrenal function after unilateral adrenal extirpation (no.=1), adrenal function after chronic cortisone therapy (no.=1). The low-dose (1-μg) ACTH test was performed by one of the laboratory physicians according to protocol. The nature of the ACTH test was explained by the laboratory physician; in addition, the patient received a brochure outlining the procedure of the ACTH test, and all patients consented to the test. For the present study, approval from the local Ethics Committee was not re-

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quired, since the ACTH test was performed in a clinical setting according to prevailing clinical and diagnostic procedures and guidelines that are in line with the Helsinki Declaration and regulations for Good Clinical and Laboratory Practice.

**Methods**

The ACTH tests were carried out at 09:00 h. An indwelling cannula (Y-CAN, Beldico, Marche-en-Famenne, Belgium) was inserted into the cubital vein for blood collection and iv injection of 1-μg tetracosectide, which was freshly prepared by diluting a 250-μg ampoule of tetracosectide (Novartis Pharma, Nurnberg, Germany) in normal saline to 1 μg in final a volume of 0.5 ml. Blood was collected before, and after 20 and 30 min of iv injection and saliva was collected by a saliva collecting tube with a cotton swab (Salivette, Starstedt, New York, NC) before and 30 min after iv injection. Blood samples were centrifuged after 30 min at 3000 rpm for 10 min, and serum was collected in plastic tubes and stored at −20 C until analyses. The salivary collecting tubes were centrifuged at 3000 rpm for 10 min and stored at −80 C until analyses, as described previously (9, 12). A normal response was defined as a total serum cortisol of >0.50 μmol/l, at 20 or 30 min, according to previously published reports (1, 13).

**Laboratory analyses**

Serum cortisol was measured by a solid-phase competitive chemiluminescent enzyme immunoassay (IMMULITE 2000 Cortisol, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with intra- and inter-assay coefficients of variation (CV) of <10%, and salivary cortisol was determined by a coated tube radioimmunoassay (Spectra, Cortisol RIA, Orion Diagnostics, Espoo, Finland) with intra- and inter-assay CV of <5.5%.

**Statistical analyses**

Data were presented as mean (SD) or as median [interquartile range (IQR)] in case of non-normally distributed variables, or as percentages. Differences between variables were tested by Student’s t-tests or by Mann-Whitney U tests, as appropriate. The relation between variables was expressed as correlation coefficients according to Spearman. The sensitivity, specificity, and area under the curve (AUC), an indicator of the predictive value of a test, were calculated. The best cut-off value was designated as the point having the maximal Youden's index (Youden's index = sensitivity + specificity – 1) (14, 15). All analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, IL). A 2-sided p-value <0.05 was considered as statistically significant.

**RESULTS**

The mean (SD) age of the patients was 49.3 (17.8) yr. Age was negatively correlated with serum basal cortisol with borderline significance (r=−0.27; p=0.054), but not to serum cortisol at 20 min and 30 min (r=−0.19; p=0.19 and r=−0.13; p=0.36, respectively). Furthermore, age was not significantly correlated with basal salivary cortisol and salivary cortisol at 30 min (r=−0.11; p=0.45 and r=0.003; p=0.98, respectively). Mean basal serum cortisol of the study population was 0.44 μmol/l (SD: 0.22 μmol/l; range 0.03-1.07 μmol/l). Serum cortisol at 20 and 30 min were 0.64 μmol/l (SD: 0.21 μmol/l; range: 0.04-1.07 μmol/l) and 0.70 μmol/l (SD: 0.25 μmol/l; range: 0.05-1.26 μmol/l), respectively. Basal serum cortisol was significantly correlated with serum cortisol at 20 and 30 min (r=0.79 and r=0.72, respectively, both p<0.001). Median basal salivary cortisol was 8.4 nmol/l (IQR: 3.8-14.2 nmol/l), whereas salivary cortisol at 30 min equaled 35.9 nmol/l (IQR: 21.1-46.2 nmol/l). Basal salivary cortisol was significantly correlated with salivary cortisol at 30 min (r=0.56; p=0.001). Serum cortisol increased 1.5-fold and salivary cortisol increased 4-fold. No significant differences in serum and salivary cortisol were observed between men and women who did not use estrogens (Table 1). Women who used estrogens (no.=9) had higher basal serum cortisol as well as salivary cortisol as compared to women who did not use estrogens (Table 1). In contrast, serum cortisol at 30 min was higher in women who used estrogens compared to women who did not use oral contraceptives. No difference was found in salivary cortisol between these 2 groups at 30 min (Table 1). A salivary cortisol at 30 min >23.5 nmol/l had a sensitivity and specificity of 78.1% and 70.0%, respectively, as compared to the serum cortisol cut-off values >0.50 μmol/l which was used to indicate adequate adrenal function (Fig. 1). The AUC of the receiver operating characteristic (ROC)-curve was 0.78. The optimal sensitivity and specificity in the “chronic fatigue”-group (no.=37) were 82.8% and 62.2%, respectively, with a cut-off value of 23.1 nmol/l and AUC of 0.77.

Ten out of the 51 patients had a serum cortisol <0.50 μmol/l at 30 min [mean: 0.36 μmol/l (range: 0.05-0.49 μmol/l) with a mean basal serum cortisol of 0.21 μmol/l (range: 0.03-0.34 μmol/l)]. The mean basal salivary cortisol in those 10 patients were 3.6 nmol/l (range: 0.6-9.1 nmol/l) with a salivary cortisol at 30 min equaling 17.7 nmol/l (range: 0.7-39.6 nmol/l). When applying the salivary cut-off value of 23.5 nmol/l, 8 out of 10 patients were classified with adrenal insufficiency. One patient was diagnosed with Addison’s disease with a serum cortisol at 30 min of <0.05 μmol/l and a salivary cortisol at 30 min of 0.7 nmol/l.

**DISCUSSION**

In the present study, we compared serum and salivary cortisol after a low dose ACTH-test. Following iv administration of 1-μg ACTH, the stimulatory response of salivary cortisol was higher in comparison with total serum cortisol. These findings are in agreement with previous studies that reported a more pronounced

<table>
<thead>
<tr>
<th>Cortisol</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>No.</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Serum cortisol (μmol/l)</td>
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<td></td>
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<tr>
<td>Basal</td>
<td>0.40 (0.16)</td>
<td>0.36 (0.17)</td>
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<tr>
<td>20 min</td>
<td>0.56 (0.20)</td>
<td>0.60 (0.20)</td>
</tr>
<tr>
<td>30 min</td>
<td>0.56 (0.17)</td>
<td>0.62 (0.20)</td>
</tr>
<tr>
<td>Salivary cortisol (nmol/l)</td>
<td>8.3 (4.1-13.5)</td>
<td>7.4 (2.4-11.9)</td>
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<td>30 min</td>
<td>31.0 (21.6-48.4)</td>
<td>36.2 (17.0-40.8)</td>
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*p<0.001, *p<0.05 compared to women not using oral contraceptives.