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Diagnostic performance of salivary cortisol and serum osteocalcin measurements in patients with overt and subclinical Cushing's syndrome

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ABSTRACT

Objective: The cut-off value for salivary cortisol measurement for the diagnosis of Cushing's syndrome (CS) may depend both on the severity of the disease and the composition of control group. Therefore, we examined the utility of midnight salivary cortisol measurements in patients who were evaluated for signs and symptoms of CS or because they had adrenal incidentalomas. Because serum osteocalcin (OC) is considered as a sensitive marker of hypercortisolism, we also investigated whether OC could have a role in the diagnosis of CS.

Patients and methods: Each of the 151 patients was included into one of the following groups: (A) overt CS ($n = 23$), (B) subclinical CS ($n = 18$), (C) inactive adrenal adenomas ($n = 40$), (D) patients without HPA disturbances ($n = 70$). Patients (C + D) were used as controls. Serum, salivary and urinary cortisol, and OC were measured by electrochemiluminescence immunoassay.

Results: Group A had suppressed OC as compared to both group B and group (C + D). Serum and salivary cortisol concentrations showed strong negative correlations with OC in patients with overt CS. The areas under the curves of salivary and serum cortisol at 24:00 h (0.9790 and 0.9940, respectively) serum cortisol after low dose dexamethasone test (0.9930) and OC (0.9220) obtained from ROC aanalysis for the diagnosis of overt CS were not statistically different.

Conclusion: This study confirms the usefulness of midnight salivary cortisol measurements in the diagnosis of overt CS in the everyday endocrinological praxis. Our results suggest that OC may have a role in the diagnosis of overt CS.

1. Introduction

Many years after the initial reports of salivary cortisol determination [1] midnight salivary cortisol, along with low dose dexamethasone test (LDDST) and urinary free cortisol (UFC) measurement [2], has become a first-line laboratory method in the diagnosis of Cushing's syndrome (CS). However, there are marked differences between different studies in the sensitivity and specificity of salivary cortisol assays [3–7], which may be due to not only differences in clinical settings and the composition of control groups, but they may be also related to differences in the diagnostic performance of various laboratory methods [8] and variations in sample collection techniques [4,11]. Until nowadays, only a few studies reported the diagnostic applicability of an automated electrochemiluminescence immunoassay for the measurement of salivary cortisol [8,9].

Midnight salivary cortisol may be used with the same threshold both in inpatient and outpatient setting [10]. Although several studies have established salivary cortisol reference data in healthy non-obese volunteers [11–13], the use of a control group more closely reflecting the everyday clinical practice (high number of patients with moderate and severe obesity, diabetes mellitus, incidentally discovered adrenal masses, etc.) may probably deteriorate the diagnostic performance of salivary cortisol measurements. However, the impact of these disturbing clinical circumstances has not been accurately explored. For example, there is a paucity of reports on the diagnostic utility of salivary cortisol measurements in patients with mild [12] or subclinical CS [14] and in patients with hormonally inactive adrenal tumors [14,15]. Therefore, in the present study we examined midnight salivary cortisol and compared its diagnostic utility to other tests including morning salivary cortisol, morning and midnight serum cortisol, LDDST and UFC in a

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large number of consecutive patients evaluated because of signs and symptoms of CS or because of the presence of incidentally discovered adrenal tumors.

Bone formation, most frequently evaluated by serum osteocalcin (OC) measurement has been known to be greatly suppressed in patients with CS [16–23]. In our recently published study we found that serum cortisol was significantly correlated with bone markers in patients with active CS [22]. Based on this observation we raised the question whether bone markers, especially OC, could serve as a potential new laboratory marker of endogenous CS [22]. Therefore, our present study also aimed to evaluate the diagnostic utility of serum OC measurements as compared to that of current methods including salivary cortisol measurements.

2. Patients and methods

The study population included a total of 151 patients consecutively referred for endocrinological evaluation because of obesity/rapid weight gain, high blood pressure; livid striae, menstrual disturbances, hirsutism, osteoporosis, or because of the presence of incidentally discovered adrenal tumors. Although each patient had at least one of the listed signs/symptoms or clinical findings, only 17 patients were suspected as having Cushing's syndrome at the first visit. As part of their clinical investigation, all patients were prospectively examined for the presence of CS.

2.1. Clinical investigations and laboratory methods

Serum cortisol concentrations were measured from blood samples collected at 08:00 and 24:00 h, as well as after a low dose (1 mg) dexamethasone suppression test. For UFC measurement 24-h urine collection was performed. Salivary cortisol was sampled between 23:00 h and 24:00 h (each patient) and at 08:00 h in the morning (41 of the 151 patients). Patients with hypertension and those with incidentally discovered adrenal adenomas were screened for pheochromocytomas (urinary VMA, metanephrine and normetanephrine excretions) and for primary hyperaldosteronism (plasma aldosterone/plasma renin activity ratio). Blood sample was taken at 08:00 h for plasma adrenocorticotropic hormone (ACTH) measurements in all patients with adrenal tumors and in patients whose initial hormonal findings suggested the possibility of CS.

For the collection of salivary samples, patients were asked to keep the cotton swab under the tongue for 1–2 min and then to place it back into the plastic container according to the manufacturer's instruction (Salivette tubes, Sarstedt, Nümbrecht, Germany). Brushing teeth, smoking, eating or drinking anything but water for at least 60 min prior to sampling were prohibited. Following centrifugation, saliva samples were stored at −20 °C until analysis. 300 μl of thawed saliva samples was processed for immunoassay (Elecsys, Roche Diagnostics).

Serum, salivary and urinary cortisol as well as plasma ACTH were measured by electrochemiluminescence immunoassay according to the instructions of the manufacturer (Elecsys, Roche Diagnostics, Basel, Switzerland). The within-run precision, between-run precision and analytical sensitivity of serum cortisol immunoassay measured on Modular Analytics E170 were 1.0–2.2 CV%, 1.4–2.8 CV% and 0.018 μg/dl (0.5 nmol/l), respectively. UFC assay was performed on dichloromethane extracted samples. The normal reference range of UFC provided by the manufacturer was 100–379 nmol/24 h.

Blood samples for measurements of serum OC were collected at 08:00 h after an overnight fast. Blood sampling was avoided after low and high dose dexamethasone suppression tests and in patients receiving antiresorptive medications within 2 years. Serum OC was measured with kits from Roche Diagnostics according to the manufacturer's instructions. The normal ranges of OC were 12–41 ng/ml and 11–46 ng/ml in healthy premenopausal females and in healthy males, respectively.

Body mass index (BMI) >30 kg/m^2 was considered an index of obesity.

2.2. Establishment of patient groups for analysis

Based on the final diagnosis, each patient was included into one of the following four groups: (1) group A, patients with overt CS (n = 23), (2) group B, patients with subclinical CS (n = 18), (3) group C, patients with hormonally inactive adrenal adenomas (hence without subclinical CS) (n = 40), (4) group D, patients without HPA disturbances (hypercortisolism excluded, lack of known adrenal tumor; n = 70). Overt CS was diagnosed if the patient had the characteristic signs and symptoms of active hypercortisolism. Of the 23 patients with CS, 11 had ACTH-producing pituitary adenomas, 2 had unknown source of ACTH overproduction (neither pituitary nor ectopic source was verified), 8 had adrenal CS due to uni- (7) or bilateral (1) adrenal tumors, and 2 had ECS. All but 2 patients with overt CS underwent surgical intervention, and histological examination confirmed the ACTH-producing or adrenocortical tumor. Subclinical CS was diagnosed in patients without overt clinical signs and symptoms of CS who had at least two of the following three criteria: (1) midnight serum cortisol concentration >5.0 μg/dl; (2) plasma cortisol concentration >3.6 μg/dl after low dose dexamethasone suppression test; (3) plasma ACTH concentration <7.2 pg/ml in patients with uni- or bilateral adrenal adenomas/hyperplasias. The most frequent final diagnoses of patients in group D were simple obesity, essential hypertension, polycystic ovarian syndrome and idiopathic hirsutism.

Because of the lack of clinically significant differences in serum cortisol at 08:00 h, 24:00 h, midnight salivary cortisol, UFC and serum OC concentrations between group C and group D (see Section 3 and Tables 1A and 1B), laboratory data obtained from these two groups were combined and used as a control group for receiver–operator characteristic (ROC) analysis.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software, version 15 (SPSS 15.0, SPSS Inc., Chicago, IL). Normality of data distribution was analyzed by the Shapiro–Wilk's test. Results are expressed as the mean ± S.D. Associations between different laboratory parameters were determined by linear regression analysis. The differences in biochemical variables between patient groups were evaluated with oneway ANOVA and Bonferroni post hoc test. A value of $p < 0.05$ was considered to be significant. The diagnostic accuracy of various tests was evaluated using ROC analysis. Optimal cut-off point for each test was obtained by calculating the Youden index from ROC analysis plotting patients with overt (group A) or subclinical CS (group B) vs. patients without overt or subclinical CS (groups C + D). Significance was set at $p < 0.05$.

3. Results

3.1. Demographic and hormonal findings

The main demographic and hormonal findings in the four groups of patients are summarized in Table 1A. Statistical differences between data of the four groups and the corresponding p values are shown in Table 1B. There were more females than males in all groups, and patients in group D were younger as