ORIGINAL ARTICLE

Low positive predictive value of midnight salivary cortisol measurement to detect hypercortisolism in type 2 diabetes

Charlotte Steffensen*, Henrik H. Thomsen*,†, Olaf M. Dekkers‡,§,¶, Jens S. Christiansen*, Jørgen Rungby** and Jens Otto L. Jørgensen*

*Department of Endocrinology and Internal Medicine, Aarhus University Hospital, †Department of Medicine, Viborg Regional Hospital, Aarhus, Denmark, ‡Department of Medicine, Section Endocrinology, Leiden University Medical Center, Leiden, The Netherlands, §Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark, ¶Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands and **Centre for Diabetes Research, Gentofte University Hospital, Hellerup, Denmark

Summary

Background Hypercortisolism is prevalent in type 2 diabetes (T2D), but analytical and functional uncertainties prevail. Measurement of salivary cortisol is considered an expedient screening method for hypercortisolism, but its usefulness in the context of T2D is uncertain.

Aim To compare late-night salivary cortisol (LNSC) with the 1 mg overnight dexamethasone suppression test (DST), which was considered 'reference standard', in T2D.

Patients and methods A total of 382 unselected and recently diagnosed patients with T2D underwent assessment of LNSC and DST, and the test outcome was related to age, gender, body mass index (BMI) and haemoglobin A1c levels. We used the following cut-off values: LNSC ≤ 3·6 nmol/l and DST ≤ 50 nmol/l.

Results The median (range) levels of LNSC and DST were 6·1 (0·3–46·2) nmol/l and 34 (11–547) nmol/l, respectively. Hypercortisolism was present in 86% based on LNSC values and 22% based on DST. LNSC, as compared to DST, had the following test characteristics: sensitivity: 85% (95% CI: 7–92%), specificity: 14% (95% CI: 10–19%), positive predictive value: 22% (95% CI: 17–27%), negative predictive value: 76% (95% CI: 63–87%), and overall accuracy: 30% (95% CI: 25–34%). LNSC and DST values were not associated with haemoglobin A1c, BMI and age in this cohort of patients with T2D.

Conclusion The LNSC is characterized by very low specificity and poor positive predictive value as compared to the DST, resulting in an overall low accuracy. Further methodological and clinical studies are needed to substantiate the relevance of cortisol status in T2D.

(Received 25 January 2016; returned for revision 7 February 2016; finally revised 23 March 2016; accepted 28 March 2016)

Correspondence: Charlotte Steffensen, Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Norrebrogade 44, Aarhus, Denmark. Tel.: +45 25468379; E-mail: mariaste@rm.dk

Introduction

Testing for hypercortisolism is presently recommended only in patients with classic symptoms and signs of Cushing's syndrome (CS) or certain unusual features for age (osteoporosis and hypertension), and in patients with adrenal incidentalomas.[1]

The prevalence of hypercortisolism in patients with type 2 diabetes (T2D) may be as high as 10%, and it has been hypothesized that this represents incipient or subclinical CS, which may have therapeutic implications.[2–5] Assessment of cortisol status in T2D is therefore of potential relevance, but several methodological issues remain to be investigated.

A recent guideline recommends the following methods for initial screening for CS: late-night salivary cortisol (LNSC), 1 mg overnight dexamethasone suppression test (DST), late-night serum cortisol or urinary free cortisol (UFC).[1] However, these tests are yet to be applied or validated in patients with T2D.

The DST is a relatively simple and accepted screening test, which can be performed on an outpatient basis.[6] A cut-off value for morning cortisol level of 50–70 nmol/l has a sensitivity of 98% and a specificity of 58–80% in the diagnosis of CS.[7,8] Measurement of LNSC is an expedient and noninvasive method, based on the observation that the nocturnal decline in cortisol secretion is absent or reduced in CS,[9] which has been applied in several studies.[9–11] However, certain unresolved methodological issues remain such as the influence of age, gender, body composition and glycaemic control.

The objective of this study was to determine sensitivity, specificity and accuracy of a single LNSC with the 1 mg DST as reference standard in patients with T2D.

Patients and methods

Patient selection

The study was performed at the Department of Endocrinology and Internal Medicine at Aarhus University hospital. A total of

382 patients (150 women and 232 men, median age 62 (range 23–85) years) recruited from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) Project[12] and diagnosed with T2D after January 2009 participated in the study. Exclusion criteria were use of any kind of exogenous glucocorticoids or oestrogen containing medications, psychiatric disease, alcohol intake >14 units/week for men and 7 units/week for women, and evidence of any acute medical condition. The patients were each interviewed via phone by the first author and none reported specific Cushingoid features.

The study was approved by the Danish Regional Ethics Committee, and informed verbal and written consent was obtained from all patients.

Midnight salivary cortisol and dexamethasone suppression test

In all patients, LNSC and 1 mg DST were performed; LNSC was always performed prior to DST. LNSC was collected at home between 23:00 and 24:00 using Salivette® Cortisol, Sarstedt, Nümbrecht, Germany. The participants were informed orally and in writing how to perform the test. The salivary samples were mailed to the laboratory within 3 days and centrifuged and analysed, or frozen for later analysis. Salivary cortisol was analysed using Orion Diagnostica's SPECTRIA Cortisol radioimmunoassay (RIA). The mean interassay variation coefficient was 8·6%. The detection limit was 0·6 nmol/l. A LNSC level ≤3·6 nmol/l was considered normal.[9] Dexamethasone was administered orally at 23:00, and serum cortisol was collected the following morning between 08:00 and 09:00 h. Plasma cortisol concentration was analysed using a chemiluminescent immunoassay with a Cobas 6000 autoanalyzer at the Department of Clinical Biochemistry. A serum cortisol concentration ≤50 nmol/l after 1 mg of dexamethasone was considered a normal response.

Statistical methods

To assess the diagnostic accuracy of LNSC, the DST was used as reference test. We calculated test characteristics (sensitivity, specificity, positive and negative predictive values and overall accuracy). In a second step, we calculated test characteristics at another cut-off level of the LNSC: 10 nmol/l. A receiver operating characteristic curve (ROC) was used to evaluate the diagnostic performance of LNSC. All data are presented as median (range) unless otherwise specified. The dependent variables were log-transformed to meet assumptions of linear regression analysis. Age, BMI and haemoglobin A1c (HbA1c) were the continuous factors assessed in the statistical model. All statistics and graphics were performed with Stata 12 (College Station, TX, USA).

Results

Patient characteristics

The median age was 62 years (23–85) and the median body mass index (BMI) of 30·5 (18·0-48·8) kg/m^2 (n = 254). A total of 221 patients (87%) were overweight (BMI > 25 kg/m^2) and 55 patients (22%) were morbidly obese (BMI > 35 kg/m^2). The median HbA1c was 47 (29–112) mmol/mol (n = 379).

Test characteristics of LNSC

The median levels of LNSC and DST were 6·1 (0·3–46·2) nmol/l and 34 (11-547) nmol/l, respectively. A total of 329 of 382 patients (86%) had ≥ 3·6 nmol/l. Eighty-four patients did not suppress serum cortisol ≤50 nmol/l after DST. Considering the 84 patients as true positives, the prevalence of hypercortisolism was 22% (95% CI: 18–26%) in T2D (Table 1).

The sensitivity of LNSC was 85% (95% CI: 75–92%), and the specificity was 14% (95% CI: 10–18%). The positive predictive value (PPV) of LNSC was 22% (95% CI: 17–27%), and the negative predictive value (NPV) was 76% (95% CI: 63–87%). The overall test accuracy was 30% (95% CI: 25–34%). Increasing the LNSC cut-off value to ≤10 nmol/l yielded a sensitivity of 18% (95% CI: 10–28%) and a specificity of 85% (95% CI: 81–89%). The corresponding PPV and NPV did not change substantially (25% and 79%, respectively).

As depicted with a ROC curve (Fig. 1), the area under the curve revealed an accuracy of LNSC of 0·57 (95% CI 0·49–0·64).

Influence of age, BMI and HbA1c on LNSC and serum cortisol after DST

To evaluate the association between age, obesity and glycaemic control and cortisol levels in patients with T2D, we compared

Table 1. Number of patients with hypercortisolism (DST > 50 nmol/l) and measurements of LNSC with cut-off value ≥3·6 nmol/l and ≥ 10 nmol/l

	Hypercortisolism: DST > 50 nmol/l	No hypercortisolism DST ≤ 50 nmol/l	
LNSC ≥ 3.6 nmol/l	71	256	PPV: 21.7% (71/327)
LNSC < 3.6 nmol/l	13	42	NPV: 76.4% (42/55)
	Sensitivity: 84.5% (71/84)	Specificity: 14.1% (42/298)	
LNSC ≥ 10 nmol/l	15	44	PPV: 25.4% (15/59)
LNSC < 10 nmol/l	69	254	NPV: 78.6% (254/323)
	Sensitivity: 17.9% (15/84)	Specificity: 85.2% (254/298)	

PPV, positive predictive value; NPV, negative predictive value

cortisol levels after DST and in LNSC. Linear regression established that neither age, BMI nor HbA1c could predict LNSC or DST (Fig. 2).

There was no significant difference in cortisol levels after DST between patients with BMI < 35 kg/m^2 (n = 199) and patients with morbid obesity (n = 55) [36(18–511) *vs* 32 (14–558) (P = 0·81)].

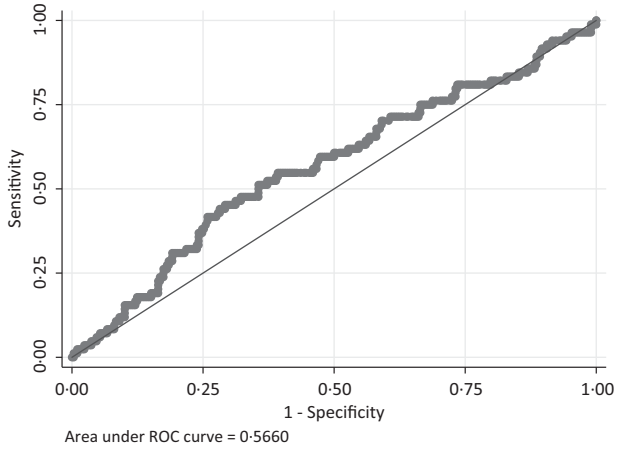

Fig. 1 The area under the ROC curve (AUC) describes accuracy of LNSC compared to DST. An AUC value of 0·57 indicates very low discriminative value.

Discussion

In this study, we recorded a prevalence of hypercortisolism in T2D of 22% according to the DST (≤50 nmol/l). Using the DST as 'gold standard', the specificity of the LNSC was very low and ROC analysis showed an accuracy of only 0·57 making LNSC of limited use as screening test for hypercortisolism in patients with T2D. Age, obesity and glycaemic control did not have a clear association with cortisol levels measured with either test.

The test results from this study are compared to normative results not derived from T2D populations, which is an important caveat. The validation of a test requires knowledge of the true diagnosis in all cases, which is clearly not available in the case of CS in patients with T2D. Thus, our findings are limited by the absence of long-term follow-up on patients and a definitive gold standard test for cortisol excess in patients with T2D.

The DST was first described by Liddle in 1960[13] and is widely used. The reported cut-off values of serum cortisol range from 100 to 200 nmol/l, but a substantial minority of patients with CS exhibit normal suppression. Therefore, a cut-off value of 50 nmol/l is generally applied to obtain sensitivity.[1,14,15] Gorges

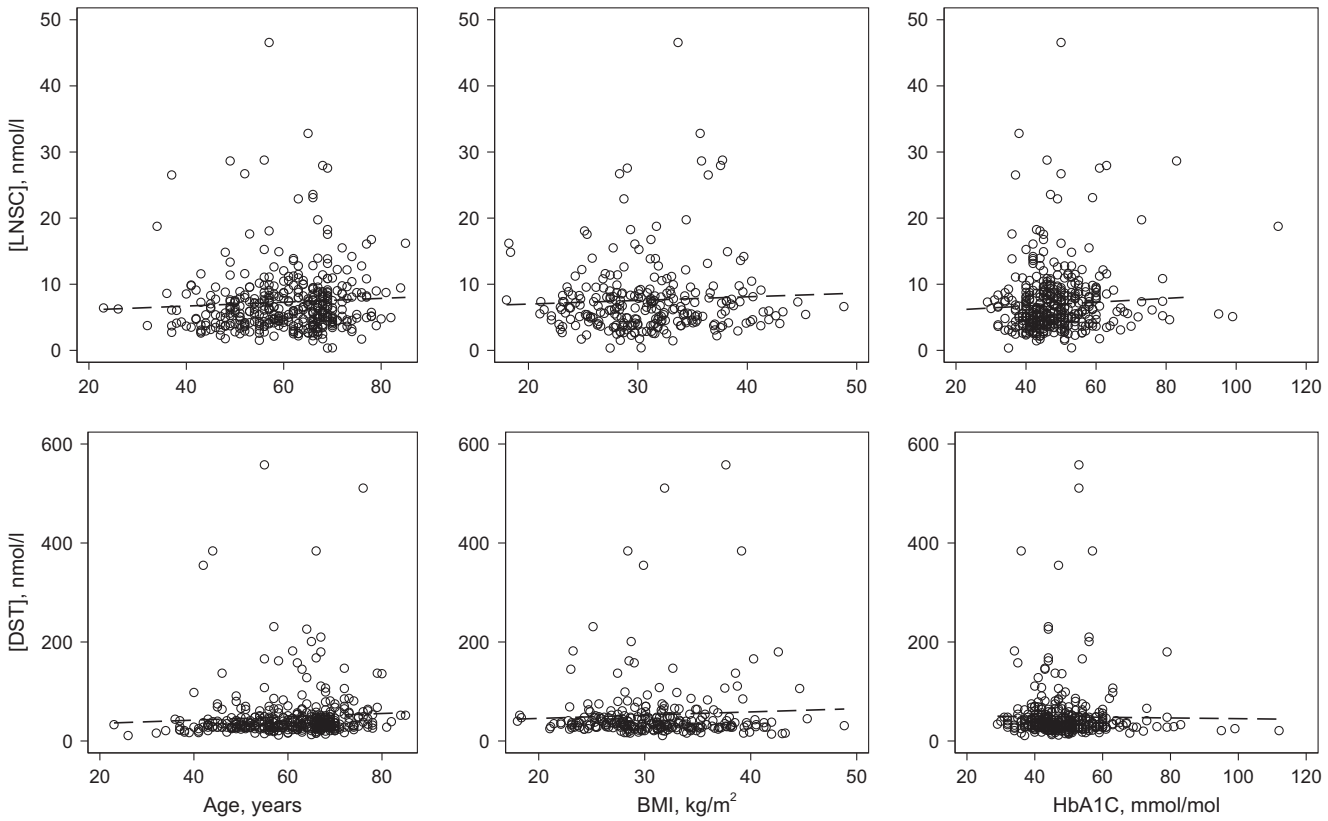

Fig. 2 Association between age, BMI and HbA1c and cortisol concentration in patients with T2D. LNSC, late-night salivary cortisol; DST, dexamethasone suppression test; BMI, body mass index. LNSC and DST are in units of nmol/l, age in years, BMI in kg/m^2. Coefficients of LNSC Age R^2 = 0·0051, P = 0·16; BMI R^2 = 0·0017, P = 0·52; HbA1c R^2 = 0·016, P = 0·014. Coefficients of DST Age R^2 = 0·0056, P = 0·15; BMI R^2 = 0·0077, P = 0·17; HbA1c R^2 = 0·0037, P = 0·24.

et al [7] tested 97 patients with CS and 101 patients without CS with a cut-off at 70 nmol/l and found a sensitivity and specificity of DST of 98% and 80%, respectively. Giraldi *et al.*[8] tested 32 patients with CS and 23 with pseudo-Cushing's states with a cut-off at 50 nmol/l and found a sensitivity and specificity of DST of 98% and 58%, respectively.

The DST has been used as screening test in previous studies of CS in T2D. Leibowitz *et al.*[16] tested 90 T2D patients with DST and observed that 4·4% failed to suppress, and 3·3% were eventually diagnosed with overt CS. A comparable study in 200 patients with T2D detected insufficient suppression in 26% and overt CS in 5·2%.[17] In a series of 289 patients with T2D, 17·6% were hypercortisolaemic after DST, and 11·9% were diagnosed with CS.[3] These figures compare well with those from the present study.

Elevated cortisol levels are also associated with obesity.[18] In a series of 269 obese patients screened with UFC, LNSC and/or 1 mg DST, no patients were finally diagnosed with CS, but 31% of the patients had at least one elevated test result.[19] In our study, however, we did not find an overall association between cortisol status and either BMI in general or morbid obesity (BMI > 35 kg/m^2) in particular.

It is also reported that chronological or biological age influences cortisol secretion.[20] Mean serum cortisol is reported to increase by 20–50% between 20 and 80 years of age in both men and women, and the nocturnal nadir also increases with age in both sexes.[21]

Poor glycaemic control has also been associated with elevated basal and stimulated cortisol levels.[22] Also, some studies suggest that T2D is caused by low-grade inflammation leading to activation of the HPA axis causing elevated cortisol levels.[23,24] In our study, we did not find any association between elevated cortisol levels and age, BMI or HbA1c; thus, the reason for the high number of patients with T2D not suppressing cortisol after DST remains uncertain.

The usefulness of measuring salivary cortisol has been described in several populations.[10,25] Hansen *et al.* have established a reference interval for salivary cortisol measured by RIA in healthy subjects, which proved to be unaffected by age, BMI, gender, smoking or days of sick leave during the past year.[26] The concentration of salivary cortisol is in equilibrium with free plasma cortisol and not influenced by the rate of salivary production.[11,27] Loss of the circadian rhythm of cortisol with absence of a late-night nadir is a consistent finding in patients with CS and is used diagnostically.[28] Measurement of LNSC constitutes an expedient and noninvasive method to detect this phenomenon.[9] When using a cut-off value of 3·6 nmol/l, which is based on normative data, we found elevated LNSC levels in ≈86% of the 382 patients with T2D. We found a sensitivity of 84·5% and a PPV of only 21% of the LNSC. Using a cut-off value of 10 nmol/l, the specificity was 85·2%, but with a sensitivity of only 19·9%. Previous studies have found a similar high number of false positives when testing with LNSC in patients with T2D.[29–31] The reason for the difference between results of LNSC and DST is unexplained, but certain factors related to salivary cortisol merit attention. Individuals using chewing tobacco or licorice containing an 11β-hydroxysteroid dehydrogenase type 2 inhibitor, which is known to convert active cortisol to inactive cortisone, may exhibit elevated salivary cortisol.[32] Elevated late-night salivary cortisol levels have also been detected in cigarette smokers.[33] Contamination of the salivary collection device with steroid containing products and blood may also influence the result.[34] Finally, mental stress immediately before the collection may also evoke an elevated salivary cortisol level.[25]

Although all of the patients in our study were instructed how to collect the salivary cortisol to avoid the above-mentioned pitfalls, it cannot be ruled out that failure to comply with the instructions prevailed and consequently gave rise to increased pre-analytical variation of the salivary cortisol measurements.

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is highly expressed in the salivary glands and rapidly inactivates cortisol by conversion to cortisone such that salivary cortisone levels are higher than those of cortisol. It has been reported that salivary cortisone levels more adequately reflect cortisol status and are detectable at low levels of serum cortisol.[35] In the present study, we did not measure cortisone levels in either serum or saliva, but this method may prove useful also as a tool to screen for hypercortisolism.

The 84 patients who did not suppress cortisol after DST are currently being followed on an outpatient basis including additional biochemical tests and imaging. At this stage, 20 patients are undergoing further examinations including plasma ACTH measurements and imaging based on continued evidence of hypercortisolism.

Collectively, we believe that the LNSC is not suitable as a stand-alone test to screen for hypercortisolism in T2D. The prognostic and therapeutic significance of the reported prevalence of hypercortisolism based on the DST in this study also remains to be further investigated. This will require rigorous long-term follow-up of patients with hypercortisolism to assess the development of overt CS. It also remains unproven whether patients with T2D who eventually develop incipient or subclinical CS will benefit from treatment of this condition.

Acknowledgements

The authors gratefully acknowledge the support and generosity of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) Project and Desirée and Niels Yde's Foundation, without which this study could not have been completed.

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