Clinical Biochemistry 46 (2013) 1442–1446

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Defining adrenal status with salivary cortisol by gold-standard insulin hypoglycemia☆

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ARTICLE INFO

Article history:
Received 15 March 2013
Received in revised form 26 April 2013
Accepted 3 May 2013
Available online 14 May 2013

Keywords:
Adrenal function
Adrenal insufficiency
Salivary cortisol
Free cortisol
Insulin hypoglycemia test

ABSTRACT

Background: Insulin-induced hypoglycemia (IHT) is considered the gold standard test for evaluating the HPA axis. Serum free cortisol or its surrogate, salivary cortisol as opposed to total cortisol concentrations, offers a better reflection of the activation of HPA axis. Our study aimed to derive reference ranges for the normal salivary cortisol levels in healthy patients and patients with adrenal insufficiency.

Design and methods: Serum cortisol concentrations, using the gold standard of IHT, and salivary cortisol were obtained. 36 patients referred to our outpatient endocrine testing unit for evaluation of adrenal function were included in the study. Most subjects had a history of suspected hypothalamic/pituitary disease causing adrenal insufficiency.

Results: We found a strong linear correlation between the serum and salivary cortisol concentrations in simultaneously collected samples ($r = 0.81$, 95% CI 0.74–0.86, $p < 0.0001$). The corresponding salivary cortisol equivalent to a serum cortisol of 500 nmol/L, using a linear-regression equation, was 16.7 nmol/L (95% CI 13.3–20.1 nmol/L, $p = 0.0001$). A salivary cortisol of 13.3 nmol/L has a specificity of 89.3% to detect abnormal HPA function. Using the upper 95% CI result of salivary cortisol 20.1 yields a sensitivity of 87.5%.

Conclusion: With the present assay, adrenal insufficiency may be diagnosed with reasonable confidence if a random salivary cortisol is lower than 13.3 nmol/L and excluded if a random salivary cortisol is higher than 20.1 nmol/L. Future studies should correlate these thresholds with clinical outcomes.

Introduction

The insulin-induced hypoglycemia test (IHT) is considered the "gold standard" in evaluating patients with suspected primary or secondary adrenal insufficiency [1]. Hypoglycemia is a strong stimulus of the whole hypothalamus–pituitary–adrenal (HPA) axis by increasing CRH release and subsequently, increasing ACTH and cortisol secretion. IHT therefore measures the integrity of the entire HPA axis and its ability to respond to physiologic stress. A normal response is considered a peak serum cortisol level greater than 497–552 nmol/L [1].

Clinicians are often asked to assess patients for possible adrenal insufficiency and yet the IHT is unsuitable or even contraindicated in certain patient populations like the acutely ill, hospitalized patient. In practice, the ACTH stimulation test is a widely used tool in interpreting adrenal insufficiency; however, this test is far from perfect. In particular, the ACTH stimulation test only reveals the ability of the adrenal gland to respond to ACTH. In settings where the clinical adrenal insufficiency is due to acute lack of CRH or ACTH, the ACTH stimulation test is often invalid or at least very difficult to interpret [2]. It is therefore crucial to use reference ranges that are independent of the level of HPA injury and derived from a gold standard test. In all cases, the actual number generated from a cortisol assay may vary according to assay in use which also underlines the importance of local validation of any test involving cortisol measurement [3].

Commonly used assays that measure serum total cortisol (serum free cortisol plus the protein-bound fraction of the cortisol) can be misleadingly lower than anticipated, resulting in the incorrect conclusion that adrenal function is impaired. Serum free cortisol measurements are not widely available in most clinical centers, so at present there is no practical way for clinicians to interpret cortisol levels in patients. Salivary cortisol concentration represents an excellent indicator of the plasma free cortisol concentration [4,5]. It is readily available in many labs and has the practical advantage of being a simple and non-invasive collection.

Most of the research on salivary cortisol measurements has been focused on its utility in the evaluation of patients with suspected Cushing's syndrome. The medical literature on salivary cortisol in the setting of adrenal insufficiency is still scant. Currently, there are no uniformly recognized, gold standard derived reference values for

Abbreviations: IHT, insulin-induced hypoglycemia test; HPA, hypothalamus–pituitary–adrenal.

☆ Funding: Study funded by the Division of Endocrinology, University of Calgary.

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0009-9120/$ – see front matter
http://dx.doi.org/10.1016/j.clinbiochem.2013.05.004

salivary cortisol in patients undergoing an IHT for suspected adrenal insufficiency.

Materials and methods

Insulin-induced hypoglycemia tests are routinely performed in our Endocrine Testing Unit at Foothills Medical Center according to a published protocol [6]. Such tests have a high degree of reproducibility and are ordered by the staff endocrinologists for the evaluation of patients with suspected adrenal insufficiency due to disorders of the hypothalamus, pituitary or adrenals [7]. Patients whose endocrinologists had deemed this test clinically necessary were approached for inclusion in the study after they had been referred to the central testing facility. Patients were excluded from participation if they were unable to give informed consent or unable to produce a salivary sample.

The insulin hypoglycemic protocol was conducted as follows: patients arrived at the testing unit in the morning after an overnight fast. Subjects were permitted to drink water prior to arrival at the testing unit. Any chronic glucocorticoid therapy was withheld for at least 24 h prior to the test or substituted to dexamethasone under the supervision of the attending endocrinologist. Intravenous access was secured and maintained with an infusion of 0.9% normal saline at 30 cc/h. Blood samples were drawn at times 0, 40, 60 and 90 min following an IV push of Regular Human Insulin 0.1 units/kg body weight at the 0 minute mark. A sample for plasma glucose alone was also drawn at the 20 minute mark. Samples were sent for measurement of plasma glucose and cortisol. Hypoglycemia was also confirmed at the bedside using a standard capillary glucose meter. After confirmation of hypoglycemia, subjects were permitted to drink a clear glucose containing liquid (apple juice) to relieve the symptoms but then did not drink further during the test.

Serum albumin was measured at baseline in each subject. Given that the rate of equilibrium of serum cortisol and salivary cortisol is estimated at less than 5 min [8], salivary cortisol was collected at each time point as serum cortisol. To do so, subjects were asked to chew on a cotton swab for 2 min before placing the swab in a test tube which was then time-labeled and transferred immediately to the laboratory.

Serum albumin and glucose were assayed by dye-binding and enzymatic-colorimetric methods on an automated general chemistry analyzer Roche Modular Analytics SWA, using the manufacturer's protocols (Roche Diagnostics GmbH, Mississauga, ON). Serum cortisol by a competitive electrochemiluminescence immunoassay on an automated clinical chemistry analyzer Advia Centaur analyzer using the manufacturer's protocols (Siemens Healthcare Diagnostics, Mississauga, Ont, Canada). The within-run CV was 4.1% and between-run CVs were 6–7% over the reportable range of the assay. Saliva was collected in Sarstedt Cortisol-Salivette containers (51.1534.500) and the cortisol determined using a commercial I125-radioimmunoassy (Coat-a-Count Cortisol from Siemens Medical Solutions Diagnostics, Mississauga, ON). The within-run CV was 4.5% for salivary cortisol level of 5.3 nmol/L. During the period of this study there were no commercially available controls for salivary cortisol. In house laboratory patient pools were prepared, aliquots made and frozen at −70 C for routine QC use. Between-run CVs established on two levels of patients specimen pools were 9.2% and 6.6 % for salivary cortisol levels of 5.9 nmol/L and 56.7 nmol/L respectively. The lower detection limit of this assay was 1.3 nmol/L.

At each time point for the test, the degree of correlation between salivary and serum cortisol samples was tested by Spearman's rank test. The gold standard normal serum cortisol response to hypoglycemia was defined as any value greater than 500 nmol/L seen at any time point following hypoglycemia. Subjects were thus classified as normal or abnormal by whether they achieved a peak serum cortisol higher than 500 nmol/L. A t-test for comparison was used to determine whether the peak cortisols (salivary and serum) were different in the groups who "failed" or "passed" the insulin hypoglycemic test by this cut-off.

Using all paired serum and salivary samples, a linear regression equation was derived to estimate the mathematical formula for conversion of a salivary cortisol result to a serum cortisol equivalent, including an estimate of the 95% confidence intervals for the y-intercept in order to be able to estimate the 95% confidence intervals for such a conversion.

Using the salivary cortisol calculated equivalent (and 95% confidence intervals) to a serum cortisol of 500 nmol/L, we used the gold standard serum cortisol results to derive the sensitivity, specificity and positive and negative likelihood ratios for a diagnosis of adrenal insufficiency based upon each subject's peak salivary cortisol levels transformed to a serum cortisol equivalent. Finally, a ROC curve analysis was performed to assess the variable sensitivity and specificity of differing cut-points of salivary cortisol in the diagnosis of hypoadrenalism.

Statistical tests were performed using commercially available software (Analyse-it for Excel v. 2.08 and Accumetric Test Performance Analysis 1.1, Accumetric Corporation, Montreal Canada). The research protocol was approved by the local institutional research review board and all subjects gave signed consent to participate.

Results

Of the 36 subjects recruited, 14 were female and 22 were male and mean subject age was 45.3 yrs (range 20–70). All subjects had a serum albumin greater than 38 g/L. The reason for referral to insulin hypoglycemic testing was as follows: pituitary tumor (50%), post traumatic brain injury (25%), suspected primary adrenal insufficiency (8.3%), suspected hypopituitarism (5.5%), chronic prednisone use (2.7%), intracranial mass (2.7%), suspected isolated ACTH deficiency (2.7%), fatigue (2.7%). Of all insulin hypoglycemic tests, 78% successfully achieved a plasma glucose less than 2.5 mmol/L. Of the 22% of tests where this hypoglycemic threshold was not achieved, only one subject failed to nonetheless achieve a serum cortisol greater than 500 nmol/L and in that single case, the peak cortisol was 475 nmol/L. In all, 8 subjects (22.3%) would be considered to have "failed" the insulin hypoglycemic test and thus 28 subjects could be definitively considered as adrenally sufficient and thus used to define a normal salivary cortisol response to hypoglycemia.

A total of 144 salivary samples were received for analysis of which only 8 were deemed to be of insufficient volume for use. These eight insufficient samples were from 6 different subjects, all of whom were able to produce salivary samples for their other time point collections. Table 1 shows the patient demographics and results classified according to diagnosis. Fig. 1 shows the correlations between paired serum and salivary cortisol samples and from the graph it may be

Table 1
Mean serum and salivary cortisol levels classified by diagnoses of hypoadrenalism.

	Normal serum cortisol response to hypoglycemia	Abnormal serum cortisol response to hypoglycemia
Number (n)	28	8
Age, mean, range (yrs)	43.1 (20–66)	53.1
Diagnoses	14 pituitary tumor 8 head injury 5 adrenal insufficiency 1 diagnosis not specified	8 pituitary tumor 1 chronic steroid use 1 head injury 1 primary adrenal insufficiency
Peak serum cortisol (nmol/L), mean, 95% CI	623 (589–658)	352 (254–450)
Peak salivary cortisol (nmol/L), mean, 95% CI	23.9 (19.4–28.3)	12.0 (5.0–15.4)

seen that a very tight correlation exists with an R-statistic of 0.81 (0.74–0.86), $p < 0.0001$. Fig. 2 shows the individual time point correlations between serum and salivary cortisol samples, each level of agreement (R-statistic) being between 0.71 and 0.91, all $p < 0.001$.

Among subjects who had a normal serum cortisol response to hypoglycemia, the mean peak cortisol was 623 (SD 88.9) nmol/L and the mean salivary cortisol was 23.9 (SD 11.4) nmol/L. Among subjects who had an abnormal serum cortisol response, the mean peak cortisol was 352 (SD 117 nmol/l, $p < 0.0001$ vs. normal serum cortisol group) and mean peak salivary cortisol 12.0 (SD 7.6 nmol/L, $p < 0.01$ vs. peak salivary cortisol in normals).

The linear regression model relating salivary cortisol to serum cortisol yielded the following equation: *salivary cortisol = (0.048 × serum cortisol) − 7.3.* with 95% confidence intervals of the y-intercept of (−3.8, −10.7). Thus, a salivary cortisol of 16.7 nmol/L (95% confidence intervals 13.3–20.1) would be the calculated equivalent to a serum cortisol of 500 nmol/L. The ROC curve analysis for salivary cortisol (not shown), showed an area under the curve of 0.79 (0.60–0.97), $p < 0.01$ vs. AUC of 0.50.

Using the serum cortisol results as the gold standard, the sensitivity, specificity and likelihood ratios of the transformed salivary cortisol results are shown in Table 2, which includes calculations of transformed salivary cortisol using the mean and 95% confidence intervals for y-intercept. This table shows that overall, a salivary cortisol of 16.7 nmol/L has generally poor sensitivity and specificity for true adrenal insufficiency. However, by using a cut-off of 20.1 nmol/L for salivary cortisol, sensitivity improves to 87.5% and by using a salivary cortisol cut-off of 13.3 nmol/L, specificity improves to 89.3%. Table 3 shows the numeric ROC analysis for both low and high pre-test probabilities of disease, demonstrating the potential usefulness of a multi-cut-point approach to interpretation of salivary cortisol results.

Discussion

This study demonstrates that the salivary cortisol response to insulin induced hypoglycemia tightly parallels the serum cortisol response at each time point during this test of adrenal function. The degree of direct correlation between paired measurements of serum and salivary cortisols are virtually identical to the results reported by other investigators [5,9,10], indicating that despite different cortisol assays, the salivary cortisol may be used as a surrogate measure of circulating cortisol. Some of the inherent variation in the correlation may be explained by the estrogen status of the patient via its effect on cortisol binding globulin and subsequent measure of serum total cortisol, however the magnitude of this effect is probably very small as shown in a study that stratified patients by estrogen therapy [11]. As well, some of the variation may be explained by a greater rise in free (salivary) cortisol following saturation of cortisol binding globulin during a stimulation test [12]. As such, future studies using free cortisol measurements may prove to be more accurate and superior to serum cortisol measures during standard adrenal stimulation tests.

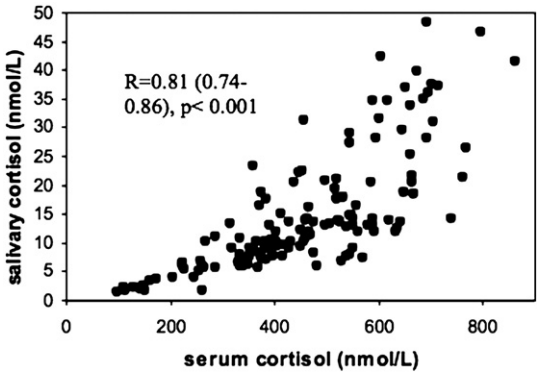

Fig. 1. Spearman rank correlation between all paired salivary and serum cortisol levels in normoalbuminemic subjects (n = 136 samples).

Our study is unique in that it is the first study to truly employ the gold standard test of adrenal function, the insulin hypoglycemic test. Previous studies have used either the 1 μg [11] or 250 μg [12] ACTH stimulation test which is itself recognized as a surrogate for the insulin hypoglycemic test. Thus, our results could be considered as the first step toward defining a gold standard for outpatient salivary cortisol measures of adrenal function. Even so and considering differing assays in use, our results showing a mean peak salivary cortisol in hypoadrenals vs. normals of 12.0 and 23.9 nmol/L are still very similar to those reported during ACTH stimulation tests, where the mean peak salivary cortisol was 9.16 and 27.6 nmol/L for hypoadrenal and normal subjects respectively [11].

It is important to define a normal reference range for stimulated salivary cortisol for several reasons. Salivary cortisol is an inexpensive and less invasive means of cortisol measurement [9]. More importantly, it may be superior to serum cortisol in assessment of the hypothalamic–pituitary–adrenal axis in hypoproteinemic patients [13]. The problem of interpreting serum cortisol in the hypoalbuminemic patient has been extensively discussed [2,14] and until serum free cortisol measures are more widely available, salivary cortisol may be a very viable option. It has been pointed out that performance of a gold standard insulin hypoglycemic test in a critically ill patient would be unadvisable [2] thus our study provides some guidance as to what reference ranges could be used, at least with this particular salivary cortisol assay.

The mass of data collected by our study and others suggests that there may not exist a single "cut-off" for defining adrenal function. The peak cortisol response to stress in health and disease will always be a spectrum and regardless of the test used, there is bound to be some possible overlap between health and partial or early adrenal insufficiency. For years, endocrine textbooks have claimed that the cut-off of "normal" vs. "abnormal" cortisol levels in a stimulation test is defined at 500 or 550 nmol/L [15,16]. It has been admitted that this exact cut-off is somewhat arbitrary but felt to be the lowest limit of the normal response to stress [1,17]. The situation in critical illness is even more chaotic with more than 5 published definitions of "normal" adrenal function [18–22]. A classic study that was used to define such normal values for non-critically ill patients showed that healthy patients may actually have stimulated cortisol levels as low as 400 nmol/L and that classically diagnosed hypoadrenal patients may still produce cortisol up to 240 nmol/L [23]. By virtue of being a small study, this experiment cannot account for the interpretation of peak cortisols that fall between 240 and 400 nmol/L. No doubt some such patients would have partial hypoadrenalism and some may actually be clinically normal, at least as defined by freedom from any future adrenal crisis or clinical manifestations.

Based upon some of the vagaries of a true gold standard laboratory diagnosis, the importance of incorporating the clinical picture and the importance of never missing a true diagnosis of hypoadrenalism, we suggest a "multi-cut-point" approach to biochemical diagnosis. A random or stimulated salivary cortisol > 20.1 nmol/L would virtually exclude adrenal insufficiency in patients for whom the pre-test probability was less than 40%. Similarly, a stimulated value of <13.3 nmol/L would be highly suggestive of a true diagnosis even if the pre-test probability was only between 20 and 30%. Obviously, salivary cortisol results far greater or less than these cut-offs would be even more convincing for clinical management.

Deutschbein et al. also suggested the use of two cutoff points when interpreting salivary cortisol levels [24]. In their study, 77 patients with proven HPA axis disease were compared to 184 controls. The authors used an upper cutoff of >21 nmol/L to confirm adrenal sufficiency and a lower cutoff of <5 nmol/L to confirm adrenal insufficient patients. Although our cutoff points are consistent with that

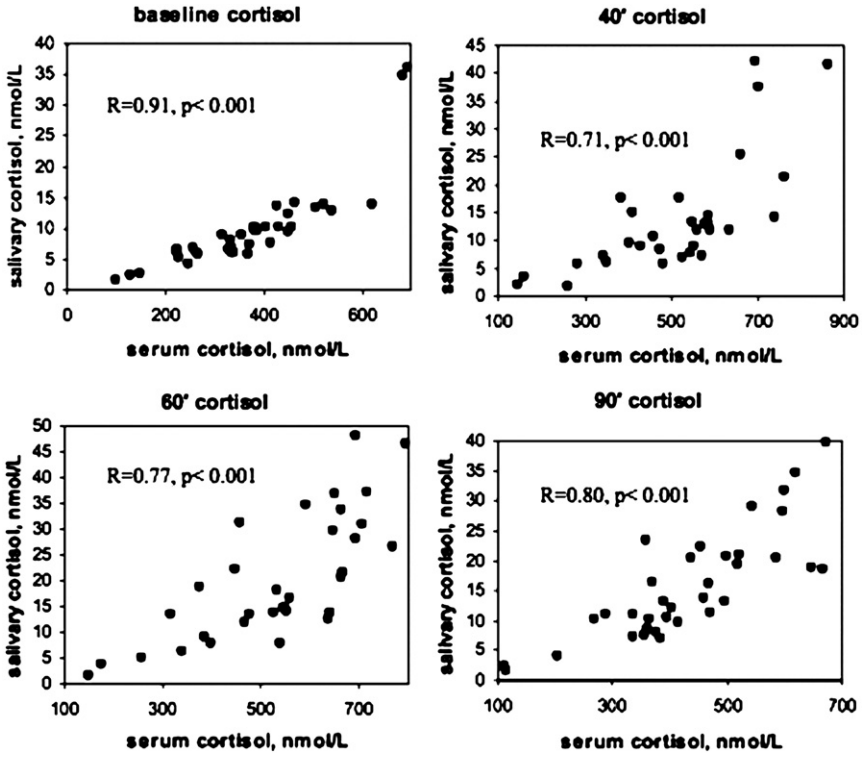

Fig. 2. Spearman rank correlations between salivary and serum cortisol at individual time points of measurement post insulin-induced hypoglycemia.

group, our exact values differ. The collections in the former study were done between 0800 h and 1000 h, however, the insulin tolerance test was not necessarily measured on the same day as the baseline salivary and serum cortisol levels. In our study however, we time matched each insulin tolerance test measurement with a salivary cortisol measurement, producing a more robust set of data for analysis.

Our study conclusions are limited by the small number of subjects; it would be preferable to validate this study with a much larger sample of both healthy and hypoadrenal subjects if the results are to be used widely. Additionally, as alluded to above, until a critical illness-specific, outcomes-based reference range is developed, our results continue to be dependent upon the use of healthy outpatient controls to define normality. It is unknown if the assay used in our institution is comparable across all other centers. It might be necessary to develop locally validated cut-off values for other assays. Lastly, we recognize the need to further confirm the validity and clinical utility of our results. A prospective study providing longitudinal outcome or follow up data would be the next logical step in further assessing the usefulness of salivary cortisol to define adrenal insufficiency.

Table 2
Diagnostic properties of salivary cortisol results expressed as serum cortisol equivalents in the diagnosis of hypoadrenalism.

Salivary cortisol (nmol/L)	16.7	13.3	20.1
Relationship to mean serum cortisol by regression equation	Mean of y-intercept for serum cortisol of 500 nmol/L	Lowest 95% confidence interval for y-intercept	Highest 95% confidence interval for 7-intercept
Sensitivity	62.5%	50%	87.5%
Specificity	61.5%	89.3%	53.5%
Positive likelihood Ratio	1.62	4.66	1.88
Negative likelihood ratio	0.6	0.55	0.23

Routinely the measurement of salivary cortisol has been mostly by immunoassay methods (IA), radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), electrochemiluminescent assays (ECLIA) and more recently by liquid chromatographic methods coupled with mass spectrometry (LCMSMS) [25]. Immunoassay methods offer the

Table 3
Differential post test probabilities for a diagnosis of hypoadrenalism based upon various degrees of pre-test probability.

Pre-test probability	Cut-point of salivary cortisol (nmol/L)	Post-test probability for true disease if less than cut-point	Post-test probability for true disease if greater than cut-point	Clinical action
0.1 (low)	7.6	1.00	0.07	If < 7.6, diagnosis confirmed
	13.1	0.34	0.06	If < 13.1, diagnosis still possible
	20.5	0.15	0.03	If > 20.5, diagnosis very unlikely
	23.3	0.15	0	If > 23.3, diagnosis excluded
0.5 (high)	7.6	1.0	0.47	If < 7.6, diagnosis confirmed
	13.1	0.82	0.34	If < 13.1, diagnosis highly likely
	20.5z	0.62	0.21	If > 20.5, diagnosis less likely but still possible
	23.2	0.62	0	If > 23.2, diagnosis excluded

convenience of improved test formats and are easily suited to automation. The additional features are unattended operation, automatic re-runs, on-board dilution as needed, and the capability of automated reflex testing based on user defined criteria. This results in better assay performance than manual radioimmunoassay which is difficult to automate. Significant advantages of ECLIA are its very sensitive enzyme-amplified chemiluminescence detection signal, achieving a wide and dynamic analytical measuring range, making these methods more amenable for salivary cortisol. The analytical sensitivity varies between immunoassays methods due to the potential for cross-reactivity with other steroids [1]. Salivary cortisol levels are generally 10 to 100-fold lower than total serum levels and can be close to the functional limit of detection for some of these immunoassays. Use of LC-MSMS have been used to improve reproducibility and accuracy, however a limited but increasing number of clinical laboratories with LC-MSMS equipment and expertise. With wider implementation, LC-MSMS measures of salivary cortisol may prove more accurate in clinical use of cortisol determinations in the future.

In summary, we have demonstrated a good correlation between paired salivary and serum cortisol measures and suggested that a gold standard, healthy outpatient peak salivary cortisol range is defined as greater than 20.1 nmol/L with decent sensitivity to otherwise detect possible adrenal insufficiency.

Acknowledgments

The authors would like to thank our endocrine testing nurse Ms. Brenda Polzin for her expert assistance in performing the insulin hypoglycemic tests.

References

[1] Erturk E, Jaffe C, Barkan A. Evaluation of the integrity of the hypothalamic–pituitary–adrenal axis by insulin hypoglycemia test. J Clin Endocrinol Metab 1998;83:2350–4.
[2] Arafah BM. Hypothalamic pituitary adrenal function during critical illness: limitations of current assessment methods. J Clin Endocrinol Metab 2006;91:3725–45.
[3] Deutschbein T, Pedersenn S. Screening for Cushing's Syndrome:New immunoassays require adequate normative data. Horm Metab Res 2013;45:118–23.
[4] Laudat M, Cerdas S, Fournier C, Guiban D, Guilhaume B, Luton J. Salivary cortisol measurement: a practical approach to assess pituitary–adrenal function. J Clin Endocrinol Metab 1988;66:343–8.
[5] Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. Clin Endocrinol 2005;63:336–41.
[6] Dons R. Endocrine and metabolic testing manual. 3rd ed.LLC, USA: CRC Press; 1998.
[7] Nye EJ, Grice JE, Hockings GI, Strakosch CR, Crosbie GV, Walters MM, et al. The insulin hypoglycemia test: hypoglycemic criteria and reproducibility. J Neuroendocrinol 2001;13:524–30.
[8] Vinning R, McGinley R, Macksyvitis J, Ho K. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem 1983;20:329–35.
[9] Wong V, Yan T, Donald A, McLean M. Saliva and bloodspot cortisol: novel sampling methods to assess hydrocortisone replacement therapy in hypoadrenal patients. Clin Endocrinol 2004;61:131–7.
[10] Contreras L, Arregger A, Tumilasci O, Persi G, Zucchini A, Cardoso E. Salivary steroids in response to ACTH. Endocronologist 2006;16:30–5.
[11] Marcus-Perlman Y, Tordjman K, Greenman Y, Limor R, Shenkerman G, Osher E, et al. Low-dose ACTH (1 μg) salivary test: a potential alternative to the classical blood test. Clin Endocrinol 2006;64:215–8.
[12] Suri D, Moran J, Hibbard JU, Kasza K, Weiss RE. Assessment of adrenal reserve in pregnancy: defining the normal response to the adrenocorticotropin stimulation test. J Clin Endocrinol Metab 2006;91:3866–72.
[13] Arafah BM, Nishiyama FJ, Tlaygeh H, Hejal R. Measurement of salivary cortisol concentration in the assessment of adrenal function in critically ill subjects: a surrogate marker of the circulating free cortisol. J Clin Endocrinol Metab 2007;92:2965–71.
[14] Knapp P, Arum S, Melby J. Relative adrenal insufficiency in critical illness: a review of the evidence. Curr Opin Endocrinol Diabetes Obes 2004;11:147–52.
[15] Dorin RI, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. Ann Intern Med 2003;139:194–204.
[16] Loriaux D. Tests of adrenocortical function. In: Becker K, editor. Principles and practice of endocrinology and metabolism. 3rd ed. Philadelphia: Lippincott-Williams & Wilkins; 2001. p. 719–23.
[17] Tuchelt H, Dekker K, Bähr V, Oelkers W. Dose-response relationship between plasma ACTH and serum cortisol in the insulin-hypoglycaemia test in 25 healthy subjects and 109 patients with pituitary disease. Clin Endocrinol 2000;53:301–7.
[18] Rothwell P, Udwadia Z, Lawler P. Cortisol response to corticotropin and survival in septic shock. Lancet 1991;337:582–3.
[19] Soni A, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T, et al. Adrenal insufficiency occurring during septic shock: incidence, outcome, and relationship to peripheral cytokine levels. Am J Med 1995;98:266–71.
[20] Marik PE, Zaloga GP. Adrenal insufficiency during septic shock. Crit Care Med 2003;31:141–5.
[21] Annane D, Sébille V, Troché G, Raphaël JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. JAMA 2000;283:1038–45.
[22] Briegel J, Schelling G, Haller M. A comparison of adrenocortical response during septic shock and after complete recovery. Intensive Care Med 1996;22:894–9.
[23] Nelson J, Tindall D. A comparison of the adrenal responses to hypoglycemia, metyrapone and ACTH. Am J Med Sci 1978;275:165–72.
[24] Deutschbein T, Unger N, Mann K, Petersenn S. Diagnosis of secondary adrenal insufficiency: unstimulated early morning cortisol in saliva and serum in comparison with the insulin tolerance test. Horm Metab Res 2009;41:834–9.
[25] Inder WJ, Dimeski G, Russell A. Measurement of salivary cortisol in 2012 — laboratory techniques and clinical indications. Clin Endocrinol (Oxf) 2012;77:645–51.