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Low-Dose and Standard Overnight and Low Dose-Two Day Dexamethasone Suppression Tests in Patients with Mild and/or Episodic Hypercortisolism

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Abstract

We previously reported on the lack of utility of the 1 mg overnight dexamethasone (DEX) test in mild and/or periodic Cushing's syndrome, as most patients with the condition suppressed to 1 mg DEX. It is possible that a lower dose of DEX as part of an overnight DEX test might be able to distinguish between mild and/or periodic Cushing's syndrome and those without the condition. The objective of the current study is to determine the sensitivity and specificity of a 0.25 mg overnight DEX suppression test, the standard 1 mg overnight DEX suppression test, and the two-day low-dose (Liddle test) DEX suppression test with and without correction for DEX levels in patients evaluated for mild and/or periodic Cushing's syndrome. Thirty patients determined to have Cushing's syndrome by biochemical testing and 14 patients determined not to have the condition had the 0.25 mg and standard 1 mg overnight DEX suppression test and the two-day low-dose DEX suppression tests. Our results show that morning serum cortisol and cortisol/DEX ratios following an overnight dexamethasone suppression test were similar in patients with Cushing's syndrome and those not having Cushing's syndrome. However, a morning cortisol value above 7.6 μg/dl following a dose of DEX of 0.25 mg was found in 12 patients with Cushing's syndrome and none in those not having Cushing's syndrome, suggesting that a high cortisol value after this low dose of dexamethasone can indicate that further testing for Cushing's syndrome is warranted. Our data suggest that the traditional 1 mg overnight or the 2 mg/2 day DEX suppression testing should no longer be used as a screening test in patients who could have mild and/or periodic Cushing's syndrome, while the 0.25 mg dose of DEX may pick up some patients with mild Cushing's syndrome.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Keywords

Cushing's syndrome; cortisol; hypercortisolism; episodic; periodic

Introduction

Cushing's syndrome is a relatively rare disorder; however, the conscreening Cushing's syndrome is a relatively rare disorder; however, the consequences of delaying its diagnosis could be quite detrimental for the patient. Because Cushing's syndrome is less common than other conditions that mimic Cushing's syndrome, such as polycystic ovarian syndrome, depression, the metabolic syndrome or obesity, a screening test with a high sensitivity and specificity is needed to identify and exclude hypercortisolism. Following the introduction of the two day dexamethasone (DEX) suppression test by Liddle in 1960 [1], tests based on the ability of low-dose DEX to suppress pituitary-adrenal function in normal subjects, but not in patients with Cushing's syndrome, have been used to screen for hypercortisolism. Since 1965, when both Nugent et al. [2] and Pavlatos [3] simultaneously reported on the overnight DEX suppression test, this test, in which 1 mg of DEX is given orally at 23:00 or 24:00 h and a plasma cortisol level is obtained at 08:00 h the next morning, has been widely used as the initial diagnostic test for Cushing's syndrome [2–12].

To provide better sensitivity, the criteria for the cut point discriminating between patients with and without Cushing's syndrome have been reevaluated and a post-DEX morning cortisol of 50 mmol/l (1.8 μg/dl) was recommended by a frequently cited review paper [13] and important practice guideline on Cushing's syndrome [14] to provide maximal sensitivity, although its sensitivity and specificity have not been prospectively evaluated.

Most early studies evaluating the overnight DEX test used patients with severe Cushing's syndrome with quite elevated cortisol levels, for whom the diagnosis was not difficult. However, recently many patients presenting with the clinical stigmata of Cushing's syndrome, but with mild and/or periodic hypercortisolism as determined by laboratory testing, are subsequently found to have Cushing's syndrome. We recently reported that 65 of the 66 patients with Cushing's syndrome had at least one normal test of cortisol status and most patients had several normal tests [15]. These patients often have some, but not all stigmata of Cushing's syndrome and need to be evaluated with a sensitive and specific screening test. Our earlier paper [16] examining the 1 mg overnight DEX in 17 patients with mild Cushing's syndrome, showed that only three of the patients failed to suppress their 08:00 h serum cortisol to a value less than 5.0 μg/dl and even with a stringent cut point, only seven of 17 patients failed to suppress to a value less than 1.8 μg/dl. The implication of this study was that the 1 mg overnight DEX test should be used with caution to exclude patients with possible mild and/or periodic Cushing's syndrome from further workup, however, this study included DEX suppression testing ordered by outside physicians, did not have a control group that did not have Cushing's syndrome, and evaluated a relatively small number of subjects.

Based on our prior publication [16], we hypothesized that the 1 mg overnight dose of DEX dose may be too high and may lead to suppressed cortisol in patients with mild and/or

periodic Cushing's syndrome and that a lower dose of DEX might be able to better distinguish those with mild Cushing's syndrome from those without the condition. Therefore, the objective of this study was to evaluate two doses (0.25 mg and 1 mg) of the overnight DEX suppression test as well as the full low-dose DEX suppression test (Liddle test) in a group of patients that were eventually determined to have Cushing's syndrome (most of them mild and/or periodic) compared to a group of patients that were tested for Cushing's syndrome, but determined not to have it.

Subjects and Methods

Subjects: Initial clinical evaluation

The initial, clinical determination was on patients who were seen in an Endocrinology clinic and were evaluated for Cushing's syndrome by T.C.F. as part of their clinical care over a period of four years (January 2006 to December 2009). Many patients had seen other endocrinologists and either the patient or the endocrinologist suspected Cushing's syndrome and often, episodic Cushing's syndrome. Patients were considered for Cushing's syndrome if they had rapid, unexplained weight gain and associated symptoms of hypercortisolism, including adult-onset hirsutism and acne, menstrual irregularities (in women), and proximal muscle weakness. Although some patients had mild dysthymic or anxiolytic symptoms, no patient had major depression, alcoholism or other causes of pseudo-Cushing's syndrome [17]. Seven patients were taking anti-depressants at the time of diagnosis. Similarly, the diagnosis of PCOS was considered unlikely by the absence of elevated total/ bioavailable testosterone [18] and lack of anovulation.

Diagnosis of Cushing's syndrome and Cushing's disease

The biochemical evaluation for Cushing's syndrome consisted of multiple measurements for 24 h UFC (24-h urinary free cortisol) and 17OHS (24-h 17-hydroxy corticosteroids), and night-time salivary and serum cortisol measurements. The tests that diagnosed Cushing's syndrome for each patient are listed in ▶Table 1. Nighttime (23:00 ± 01:00 h of their local time) plasma cortisol determination was measured in ambulatory patients [19] during the patients' initial clinic visit and was, at times, repeated in the patients' area of residence at an urgent care or emergency room. This test was not performed in women taking oral contraceptives or oral estrogens due to the effect of these drugs on raising cortisol-binding globulin (CBG) [20, 21]. Subjects fasted for at least three hours prior to venipuncture and were seated for at least 15 min prior to venipuncture. No patients had blood drawn following an unsuccessful venipuncture that could raise cortisol levels [22]. Patients collected nighttime salivary cortisol levels and urine (simultaneously measured for both UFC and 17OHS) on different days; these tests were done in the patient's own area and were sent to Esoterix Laboratories (see below) for analysis. Most patients reported that their symptoms were more severe at certain times. Therefore, subjects were instructed to collect plasma for night-time cortisol (with the exception on their first visit), saliva for nighttime salivary cortisol levels and urine (for both UFC and 17OHS) when they had signs/symptoms of short-term hypercortisolism, including sleep disturbances, acne, increased appetite, and home self-monitoring for hypertension and hyperglycemia (in those patients with hypertension and diabetes, respectively). During their home testing for hypercortisolism,

patients also underwent DEX testing as described below. Following DEX testing, biochemical testing for hypercortisolism were not performed for a period of at least 10 days.

Patients were diagnosed with Cushing's syndrome, if they had convincing and progressive signs and symptoms of hypercortisolism coupled with two or more separate abnormal values on tests of hypercortisolism (UFC, 17OHS or 17OHS/Cr ratio, night-time salivary cortisol measurement or night time serum cortisol measurement). The number of tests performed was done on a clinical basis and was stopped if either the patient had convincing biochemical evidence of hypercortisolism to diagnose them with Cushing's syndrome or when Cushing's syndrome had been excluded. The latter was determined by finding a) another diagnosis, b) lack of progression of signs and symptoms over a one-year period or 3) lack of hypercortisolism of two or more different types of tests after a minimum of three tests in each category (UFC, 17OHS or 17OHS/Cr ratio, night-time salivary cortisol measurement) for most subjects.

The type of Cushing's syndrome was then determined by finding a non-suppressed ACTH level (excluding adrenal adenomas) after which we performed biochemical testing including DEX suppression, magnetic resonance imaging (in general, 1.5 or 3.0 T magnet with thin slices through the sella and dynamic post-contrast sequences), and if needed, bilateral inferior petrosal sinus sampling with CRH [23]. In patients found to have Cushing's disease, pituitary surgery was performed by experienced skull-base surgeons or neurosurgeons, including H.K.S in Los Angeles, I.E.M. in Houston, and other experienced surgeons in Los Angeles, San Francisco and Pittsburgh.

Retrospective evaluation

The second part of the evaluation was a retrospective review of charts of all patients seen in the clinic of T.C.F. for hypercortisolism and was performed by M.M., N.S. and M.H. The Institutional Review Board at Charles R. Drew University deemed that retrospective review of the collected data was exempt from formal Institutional Review Board review in accordance with federal regulations [45CFR 46.101(b) (4)]. To be included in the Cushing's syndrome group, subjects needed to have 1) diagnosis with Cushing's syndrome with a tumor found at surgery, 2) resolution of their clinical signs and symptoms as assessed at an in person or phone appointment between three months to one year after definitive surgery (pituitary surgery or bilateral adrenalectomy following unsuccessful pituitary surgery, that may have been performed at a later date), 3) resolution of their preoperative biochemical abnormalities as measured in the three to nine month post-operative period, and 4) completed the cortisol measurements of all 3 of the dexamethasone suppression tests. All 30 patients were included in this group were found to have pituitary Cushing's disease and all patients underwent pituitary surgery as their initial surgical treatment. Fifteen patients were cured after their first pituitary surgery, three were cured after their second pituitary surgery, 12 were cured after bilateral adrenalectomy following one unsuccessful pituitary surgery, one was cured after bilateral adrenalectomy following two unsuccessful pituitary surgeries and one was cured after bilateral adrenalectomy following three unsuccessful pituitary surgeries.

To be included in the control group, patients had to have been evaluated for Cushing's syndrome in the same clinic and had similar testing but determined thereby not to have the condition and to have undergone the cortisol determination of the three dexamethasone suppression tests. Fourteen patients met the criteria. Often the patient was diagnosed with another condition.

Medications that affect the metabolism of dexamethasone by inducing or inhibiting the cytochrome CYP3A4 enzyme were obtained from Valassi et al. [24] and extracted from the patient's records. Medication categories affect the metabolism of dexamethasone include SNRI/SSRIs, statins, calcium channel blockers, proton-pump inhibitors, benzodiazepines, ACEIs and ARBs and beta-adrenergic blockers.

Methods

Patients were instructed to start the DEX suppression testing when they had signs/symptoms of short-term hypercortisolism. For DEX suppression testing, one half of a 0.5 mg pill of DEX was given Sunday night at 00.00 h (midnight) and serum for plasma cortisol and DEX levels was collected at 08:00 h the following morning. On Tuesday night at 00:00 h (midnight), two 0.5 mg pills of DEX were given and serum for plasma cortisol and DEX levels was collected at 0800 h the following morning. On Saturday, patients took one 0.5 mg of DEX pill at 09:00, 15:00, and 21:00 h and continued taking the dose at 03:00, 09:00, 15:00, and 21:00 h and on Monday at 03:00 h. Serum for plasma cortisol and DEX levels was collected at 09:00 h.

All hormones were measured by Esoterix Endocrinology, Calabasas Hills, CA. Plasma and urinary free cortisol and DEX levels were measured by HPLC MSMS (high pressure liquid chromatography with tandem mass spectrometry detection) after solvent extraction. For 08:00 h plasma cortisol measurement, the 95 % tolerance interval (normal range) as reported by Esoterix Endocrinology is 8.0–19 μg/dl with an assay sensitivity of 1 μg/dl [25]. For DEX measurements, the 95 % tolerance interval for a morning sample after 1 mg DEX the previous evening is 145–295 ng/dl, and 1600–2850 ng/dl after 8 mg DEX (4 × 2 mg doses previous day) with an assay sensitivity of 5 ng/dl [26]. For UFC measurement, the 95 % tolerance interval was determined by measurement in 20 healthy adult females and males and found to be 10–34 μg/d for females and 11–84 μg/d for males [27]. Salivary cortisol was measured by HPLC Tandem Mass Spectrometry after collection in a saliva collection device (normal range for late night values < 0.010–0.090 μg/ dl). The 24-h urine for 17-hydroxycorticosteroids (17-OHS) (normal range for adult females 2.0–6.0 mg/d, normal range for adult males 3.0–10 μg/d) was measured as Porter–Silber chromogens following enzymatic hydrolysis and purification by solvent extraction and column chromatography on silica gel [28]. Values above the range were considered abnormal.

Statistical Methods

The receiver operating characteristic curve (ROC), which relates the false positive rate (1-specificity) to the true positive rate (sensitivity) was developed for each of the three DEX suppression test metrics (cortisol, DEX, and cortisol:DEX ratio) at the three doses of DEX considered (0.25, 1.0, and 2 mg/2 day) for a total of 9 curves. The area under the curve

(AUC) was calculated using the empirical method of DeLong et al. [29] and compared to a value of 0.5 (representing a non-informative test or one that is no better than chance) using a large sample z-test with a one-tailed p-value and a significance level of 0.05. Comparison of cortisol, DEX, and cortisol/DEX ratios among patients with Cushing's syndrome in those taking medications affecting DEX metabolism to those not taking medications affecting DEX metabolism was performed using a two-sample t-test.

Results

▶Table 1 shows the 30 patients (27 females, 3 males, all Caucasians) who were determined to have Cushing's syndrome and had all three cortisol levels after the 3 DEX suppression tests (18 of the 30 had DEX levels measured in all tests). Listed are their age, gender, BMI and abnormal tests used to diagnose Cushing's syndrome in them. The age range was 17–62 years (median 40 years) and BMI was 34.2 ± 7.0 kg/m^2 (mean ± SD). In comparison, Supplementary Table 1 shows the 13 patients (10 females, 3 males, 12 Caucasians, 1 Hispanic), who were determined to not have Cushing's syndrome and had cortisol values after the three DEX suppression tests (9 of the 14 had DEX levels measured at all tests). Listed are their age, gender, and BMI. The age range was 13–64 years (median 45 years) and BMI was 36.9 ± 8.5 kg/m^2 (mean ± SD). ▶Table 2 shows mean ± SD, number of patients and p values for the cortisol, DEX and cortisol/DEX ratios following the 0.25 mg, 1.0 mg and 2 mg/2 day DEX tests.

▶Fig. 1 shows the values of 08:00 h serum cortisol (▶Fig. 1a), DEX (▶Fig. 1b) and cortisol/DEX ratio (▶Fig. 1c) the morning following administration of 0.25 mg of DEX at 00:00 h in the two groups. Although there were no statistically significant (from an AUC of 0.50) cut points for serum cortisol or the cortisol/DEX ratio to distinguish between Cushing's syndrome (Supplementary Table 2) and not having Cushing's syndrome, a cortisol value above 7.6 μg/ dl (▶Fig. 1a) was found in 12 patients with Cushing's syndrome and none in those not having Cushing's syndrome. Using a value of 7.3 μg/dl (▶Fig. 1a Supplementary Table 2), 16 patients with Cushing's syndrome and 1 in those not having Cushing's syndrome had a value equal to our above that cut point. No clear distinction between the two groups was found for DEX levels (▶Fig. 1b) and cortisol/DEX ratio (▶Fig. 1c).

▶Fig. 2 shows the values of 08:00 h serum cortisol (**A**), DEX (**b**) and cortisol/DEX ratio (**C**) the morning following administration of 1.0 mg of DEX for the two groups. Although there were no statistically significant cut points to distinguish between the two conditions (Supplementary Table 2), four patients with Cushing's syndrome had a cortisol value after DEX above 1.8 μ/dl (▶Fig. 2a), 2 of them had a value > 4.7 μg/dl (the highest value in the non-Cushing's syndrome group), and 2 had a DEX/cortisol ratio above 20 (▶Fig. 2c), but none in those not having Cushing's syndrome. All of the patients who had a serum cortisol level above 4.7 μg/dl in the 1.0 mg DEX test also had a serum cortisol value above the 7.6 μg/dl in the 0.25 mg DEX test. The majority of patients determined to have Cushing's syndrome as well as those determined not to have Cushing's syndrome had suppressed cortisol values less than 1.8 μg/dl (▶Fig. 2a) and a DEX/cortisol ratio less than 20 (▶Fig. 2c). Surprisingly, DEX levels were lower in patients determined to have Cushing's

syndrome compared to those determined not to have Cushing's syndrome (▶Fig. 2b, ▶Table 2).

▶Fig. 3 shows the values of 09:00 h serum cortisol (**A**), DEX (**b**) and cortisol/DEX ratio (**C**) the morning following administration of 0.5 mg of DEX every six hours for 8 doses in the two groups. Although there were no cut points to distinguish between the two groups (Supplementary Table 2), two patients with Cushing's syndrome had an elevated cortisol value after DEX above 5 μg/dl (▶Fig. 3a) and a DEX/cortisol ratio above 20 (▶Fig. 2c) but none in those not having Cushing's syndrome. The majority of patients determined to have Cushing's syndrome as well as those determined not to have Cushing's syndrome had suppressed cortisol values less than 1.4 μg/dl (▶Fig. 3a) and a DEX/cortisol ratio less than 8 (▶Fig. 3c). DEX levels were similar in the two groups (▶Fig. 3b).

Supplementary Table 2 shows the summary of receiver-operator characteristics (ROC) analyses for the three DEX suppression tests. Using the established cortisol cut point for the 1.0 mg DEX test of 1.8 μg/dl, the sensitivity was 18 % and specificity was 86 %. For all three DEX tests, no cut point for either cortisol or cortisol/DEX ratio was able to statistically distinguish between patients with Cushing's syndrome and those without the condition. Using a cut point of a DEX level 235 ng/dl following the 1 mg overnight DEX test yielded a significant area under the ROC curve ($p = 0.007$) with Cushing's subjects having lower DEX levels.

▶Table 3 shows that there was no significant difference in the values for cortisol, DEX and cortisol/DEX ratios for the 0.25 mg and 1 mg DEX test, and the 2 mg/2 day DEX test among patients with Cushing's syndrome in those taking medications affecting DEX metabolism compared to those not taking medications affecting DEX metabolism. There was a trend toward lower cortisol values in the 0.25 mg DEX test and higher DEX levels in the 1 mg DEX test in those taking medications affecting DEX metabolism. There were not enough patients in the Cushing's excluded group to perform a reasonable statistical analysis.

Discussion

Summary of findings and comparison to other papers

This study challenges the use of the 1 mg overnight or the 2 mg/2 day DEX suppression tests to screen for hypercortisolism. The study found that the great majority of patients with mild and/or periodic Cushing's syndrome did not suppress to these tests and that using different cut points for cortisol or cortisol/dexamethasone ratios, we were unable to distinguish those with Cushing's syndrome from those without it. Valassi et al. [24] showed that patients taking medications affecting cytochrome CYP3A4 showed less suppression on the 2 mg/2-day DEX suppression test that was part of the DEX-CRH test than those taking not taking medications, presumably as a result of increased DEX clearance. Increased clearance may be part of the cause of lack of suppression in some patients taking these medications given DEX. Conversely, decreased DEX clearance may be a possible reason for suppression of cortisol but higher than expected DEX levels in patients on medications that inhibit CYP3A4. Overall, the DEX/cortisol ratio should take into account the effect of DEX metabolism. It is possible that the suppression to DEX seen in this study may be due to the

episodicity of the hypercortisolism in these patients and if the test were repeated during a time of hypercortisolism, less suppression might have been seen. Alternatively, the mild nature of the hypercortisolism might have also led to easier suppression by DEX. Regardless, these findings show that the 1 mg overnight or the 2 mg-2 day DEX suppression tests are inadequate as screening tests for Cushing's syndrome in subjects with mild and/or periodic Cushing's syndrome, due to a lack of sensitivity (high false negative rate).

Based on our prior publication [16], we hypothesized that the 1 mg overnight dose of DEX dose may lead to suppressed cortisol in patients with mild and/or periodic Cushing's syndrome and therefore evaluated whether a lower dose of DEX (0.25 mg given overnight) might be able to better distinguish those with the two conditions. Using this dose, a cortisol value above 7.6 μg/dl was found in 11 patients with Cushing's syndrome and none in those not having Cushing's syndrome and using a value of 7.3 μg/dl, 16 patients Cushing's syndrome and 1 in those not having Cushing's syndrome had a value equal to or above that cut point. This suggests that using either of these cut points, high specificity can be obtained, even though the sensitivity would be poor. In other words, an elevated cortisol value following the 0.25 mg overnight DEX test would make Cushing's syndrome likely and encourage further testing, while a suppressed value could not be used to exclude the condition. The 0.25 mg overnight DEX test was able to identify more patients that had Cushing's syndrome above a cut point than either the 1 mg overnight or the 2 mg/2 day DEX suppression tests (higher specificity).

Our results showing lower DEX levels following 1.0 mg DEX in the group with Cushing's syndrome compared to those without Cushing's syndrome are intriguing and suggest that patients with Cushing's syndrome might metabolize DEX faster or absorb DEX less than those without the condition. Meikle and colleagues [30] measured morning DEX levels following the 1.0 mg DEX test and found that patients with Cushings's syndrome had a non-significant lower levels of DEX (361 ± 130 ng/dl) compared to normal subjects (417 ± 110 ng/dl). However, our finding of similar DEX levels in the two groups following the 0.25 mg and 2 mg/2 day DEX test suggest that our finding of lower levels in the Cushing's syndrome group in the 1.0 mg DEX test need to be replicated in a larger study.

Findling and colleagues [31] reported that 18 % of patients with Cushing's syndrome had a morning cortisol of < 135 nmol/l (4.8 μg/dl) to the 1.0 mg overnight DEX, and 38 % of patients with Cushing's syndrome had a suppressed UFC following the 2 mg-2 day DEX test. Plasma cortisol levels were not reported in the 2 mg/2 day DEX test. The finding of lack of suppression of cortisol following the 1.0 mg overnight DEX in that study was higher than in the prior literature, but much less than the finding here that 83 % of patients with Cushing's syndrome who suppressed to overnight DEX when the cut point of greater than 5.0 μg/dl was used and 61 % when the cut point of greater than 1.8 μg/dl was used. While Findling and colleagues concluded from their data that DEX suppression should not be used as the sole criterion to exclude Cushing's syndrome, the data here confirm the lack of utility of traditional DEX testing to exclude Cushing's in patients with mild and/or periodic Cushing's syndrome, exactly the patient group in whom the diagnosis is most difficult.

Chriguer et al. [32] used three doses of overnight DEX (0.25 mg, 0.5 mg and 1.0 mg) in healthy individuals. The mean cortisol values were 198 ± 19 nmol/l (7.1 ± 7 μg/dl), 57.1 ± 9.0 nmol/l (2.0 ± 0.3 μg/dl) and 37.9 ± 2.6 nmol/l (1.4 ± 0.3 μg/dl), respectively. Our values for the 0.25 mg and 1.0 mg test were slightly higher in patients with mild Cushing's syndrome and slightly lower in patients determined to not have Cushing's syndrome, but in the same range, indicating agreement with the literature. Huizenga et al. [33] and Chriguer et al. [32] found individual differences in glucocorticoid sensitivity, which may also explain the variability in cortisol levels in following DEX in our study.

Limitations

There may have been a referral bias resulting in a particularly low sensitivity for DEX testing in diagnosing Cushing's syndrome, as patients who failed to suppress to overnight DEX tests in outside testing may have been directed by their referring physicians directly to a neurosurgeon. More likely, however, sensitivity may have been overestimated, because referring physicians who perform this test would likely discourage patients who have suppressed results from pursuing the diagnosis further. The distinction between those with Cushing's syndrome and those without it was done on clinical suspicion as well as laboratory testing, which introduces some subjectivity in the classification. Similarly, there was some subjectivity in those without Cushing's syndrome as lack of clinical progression was a factor in their exclusion. Furthermore, patients were excluded if they were diagnosed with another condition and it is possible that a patient could have the other condition plus Cushing's syndrome.

Conclusions

In conclusion, this paper provides evidence that suppressibility to the 1 mg overnight or 2 mg/two day DEX test should not be used to exclude patients suspected of having mild or episodic Cushing's syndrome. A high cortisol value following the 0.25 mg DEX test suggest that the patient may have Cushing's syndrome and should be evaluated further. Patients suspected of Cushing's syndrome should be screened with other tests such as nighttime serum or salivary cortisol measurements or UFC, as measured by chromatography, high-performance liquid chromatography (HPLC) or mass spectrometry with two different positive tests indicative of Cushing's syndrome as suggested by a recent practice guideline [14]. Evaluation of the 0.25 mg overnight DEX test in a larger number of patients with mild Cushing's syndrome as well as those in whom Cushing's syndrome was excluded may yield discrimination between the two conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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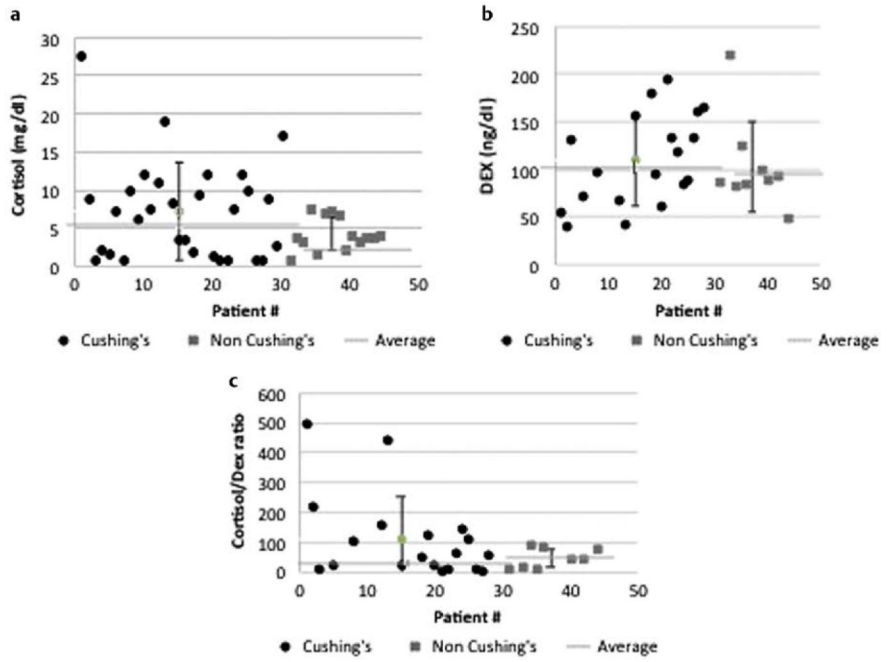
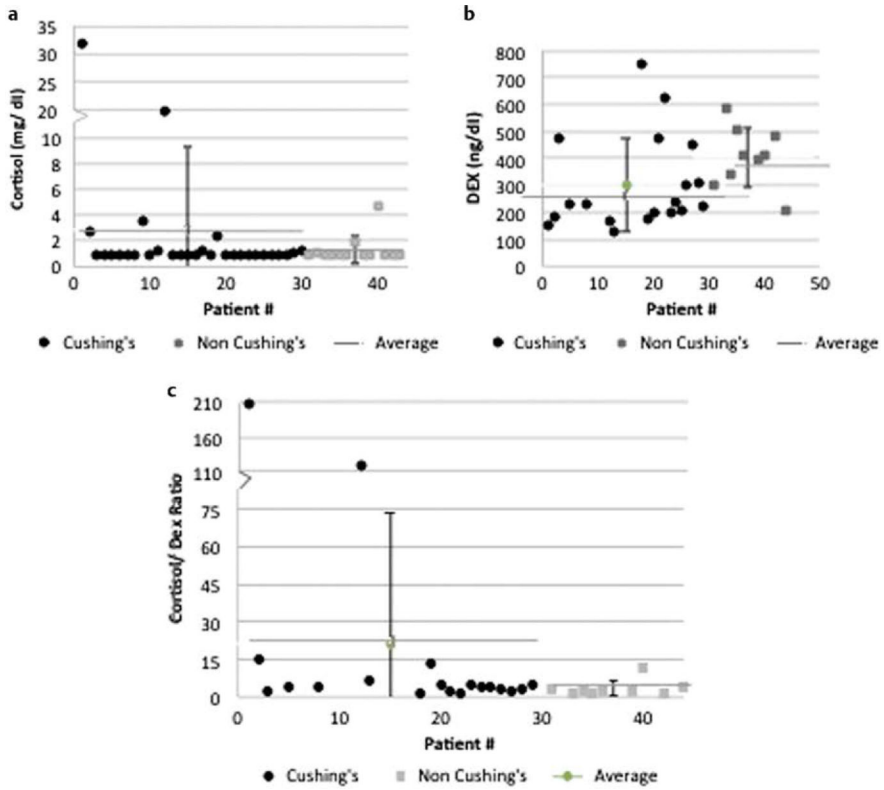


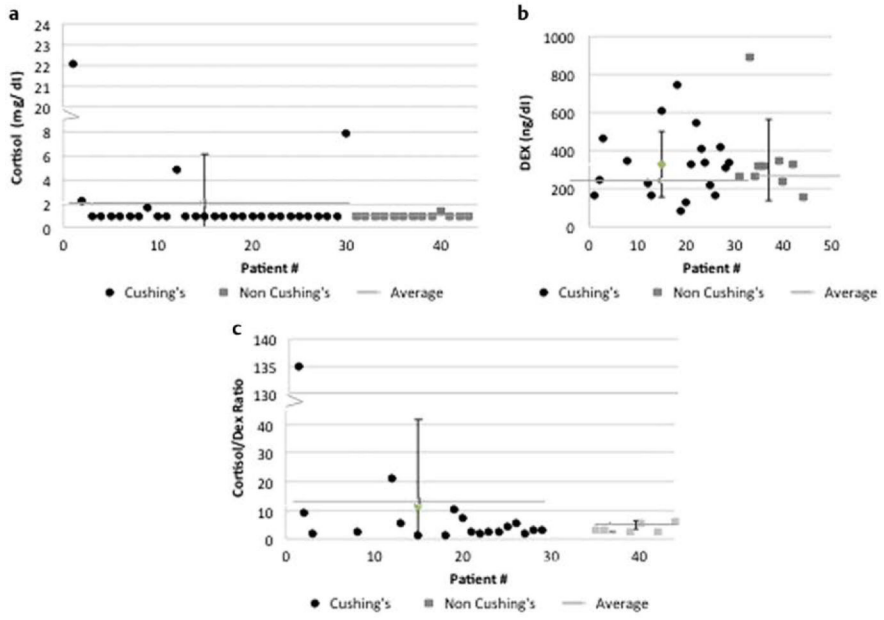
▶Fig. 1.

Values of 08:00 h serum cortisol **a**, DEX **b**, and cortisol/DEX ratio **c** the morning following administration of 0.25 mg of DEX at 00:00 h for patients determined to have Cushing's syndrome and determined not to have Cushing's syndrome. Mean and SD are depicted.



▶Fig. 2.

Values of 08: 00 h serum cortisol **a**, DEX **b**, and cortisol/DEX ratio **c** the morning following administration of 1.0 mg of DEX at 00:00 h for patients determined to have Cushing's syndrome and determined not to have Cushing's syndrome. Mean and SD are depicted.



▶Fig. 3.

Values of 09:00 h serum cortisol **a**, DEX **b**, and cortisol/DEX ratio **c** the morning following administration of 0.5 mg of DEX every six hours for two days (eight doses) for patients determined to have Cushing's syndrome and determined not to have Cushing's syndrome. Mean and SD are depicted.

▶Table 1

Patients with Cushing's syndrome with number of abnormal tests.

Patient #	Age	Gender	BMI	Nighttime plasma cortisol	Nighttime salivary cortisol	UFC	17OHS
1	30	F	23.5	1		4	
2	30	F	22.4	1		2	1
3	55	F	29.7	6		1	1
4	58	F	46.2	1		4	
5	21	F	49.1		1	3	6
6	44	M	33.4	1	3		4
7	40	F	40.5	1	1		6
8	39	F	38.9	1	2	5	
9	49	F	29.7	1	2	2	4
10	46	F	35.7	1	3		
11	40	F	34.6	1	3		1
12	62	F	25.5	1	3	4	5
13	17	M	26	1	4	1	2
14	17	F	33.7		1	2	2
15	35	F	32.4	1			3
16	49	F	41.2		3	4	> 6
17	21	F	29.2		3	1	6
18	33	F	35.9	2	3		> 6
19	41	F	43.6	1		4	2
20	50	F	33.5	1	1		
21	27	F	29.8	1		1	6
22	32	F	34.2			4	5
23	39	F	40.5	2	2		> 6
24	34	F	32.8	1		1	4
25	48	F	39.3			5	> 6
26	33	F	45.4		5	3	3
27	40	F	38.9	1	2	2	> 6

Patient #	Age	Gender	BMI	Nighttime plasma cortisol	Nighttime salivary cortisol	UFC	17OHS
28	40	F	31.2	2		2	1
29	23	F	25.4	5	5		
30	53	M	25.2		3	3	

BMI: Body mass index: UFC: 24-h urinary free cortisol; 17OHS: 24-h 17-hydroxy corticosteroids.

▶Table 2

Number of patients, mean ± SD, z-value, and p-value for the cortisol, DEX, and cortisol/DEX ratios following the 0.25 mg, 1.0 mg and 2 mg/2 day DEX tests in patients with Cushing's syndrome compared to those found not to have it.

Measurement: Test	n (Cushing's, Cushing's excluded)	Cushings's mean (SD)	Cushings's excluded mean (SD)	Z-value[1]	p-Value
Cortisol: 0.25 mg DEX	(30, 13)	7.19 (6.3)	4.20 (2.2)	−1.10	0.27
Cortisol: 1 mg DEX	(30, 13)	2.81 (6.5)	1.28 (1.1)	−0.48	0.63
Cortisol: 2 mg/2 d DEX	(30, 13)	2.04 (4.1)	0.94 (0.11)	−0.51	0.61
Dex: 0.25 mg DEX	(19, 9)	109 (48)	103 (48)	−0.42	0.68
Dex: 1 mg DEX	(19, 9)	301 (170)	405 (110)	1.97	0.049
Dex: 2 mg/2 d DEX	(19, 9)	327 (170)	346 (210)	−0.10	0.92
Cortisol:Dex ratio: 0.25 mg DEX	(19, 9)	109 (142)	46.9(34)	−0.61	0.54
Cortisol:Dex ratio: 1 mg DEX	(19, 8)	21.2 (52)	3.46 (3.1)	−1.52	0.13
Cortisol:Dex ratio: 2 mg/2 d DEX	(19, 9)	11.7 (30)	3.77 (1.4)	0.29	0.77

[1] Wilcoxon rank-sum test comparing Cushing's and Cushing's-excluded means.

▶Table 3

Effect of medications affecting dexamethasone metabolism on measurements in patients with Cushing's syndrome compared to those found not to have it.

Measurement: Test	Cushing's Meds affecting DEX n=6–10	Cushing's No meds affecting DEX n = 12–20	p-Value	Non-Cushing's meds affecting DEX n = 0–2	Non-Cushing's No meds affecting DEX n=6–11
Cortisol: 0.25 mg	4.05 (3.6)	8.76 (6.9)	0.053	4.95 (2.3)	4.06 (2.3)
Cortisol: 1 mg	1.35 (0.94)	3.55 (7.9)	0.395	0.9 (0)	1.35 (1.1)
Cortisol: 2 mg-2d	1.12 (0.47)	2.50 (4.9)	0.388	0.9 (0)	0.945 (0.12)
Dex: 0.25 mg	115 (50)	107 (48)	0.724	219	88.6 (21)
Dex: 1 mg	398 (220)	245 (110)	0.060	585	382 (97)
Dex: 2 mg/2 d	415 (210)	287 (140)	0.136	891	278 (62)
Cortisol:Dex ratio: 0.25 mg	53.0 (85)	135 (160)	0.253	15.1	51.5 (34)
Cortisol:Dex ratio: 1 mg	4.36 (4.7)	31.1 (64)	0.292	1.54	3.70 (3.3)
Cortisol:Dex ratio: 2 mg/2 d	3.79 (3.2)	15.4 (36)	0.452		3.77 (1.4)

Values are Mean (SD). In the No-Cushing's Meds affecting DEX, there was only 1 or 0 values, so SD was not calculated. *t*-Test was performed between Cushing's Meds affecting DEX and the Cushing's No meds affecting DEX with the p-value listed in column 4. Because of the small number of patients, no *t*-test was performed in the non-Cushing's patients.