# Comparison of Efficacy between 1 mg Overnight Dexamethasone Suppression Test and 2-Day Low Dose Dexamethasone Suppression Test for Diagnosis of Cushing's Syndrome

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***Objective:*** *To assess the sensitivity and specificity of 1 mg overnight dexamethasone suppression test (ODST) and the 2-day low dose dexamethasone suppression test (LDST) for diagnosis of Cushing's syndrome, and to compare the accuracy of both tests using cutoff value at below 5 μg/dl and at below 1.8 μg/dl.*
***Material and Method:*** *The present study is a retrospective study, from 1971-2007, in one academic center of 77 patients with clinical suspicion of Cushing's syndrome. Kappa statistical analysis was used to determine agreement between the two tests. Sensitivity and specificity of the tests were calculated. ROC curves were created to determine the best cutoff value of the two tests.*
***Results:*** *ODST has very good agreement with the more troublesome LDST and has comparable efficacy. Lowering the cutoff value from 5 μg/dl to 1.8 μg/dl does not improve the accuracy of ODST but results in decreased specificity of LDST. The best cutoff value of ODST test is ≥ 5.3 μg/dl and the best cutoff value of LDST is ≥ 5 μg/dl.*
***Conclusion:*** *ODST is an efficient method for diagnosis of patients suspected of having Cushing's syndrome. The attempt to lowering cutoff value does not improve the efficacy of dexamethasone suppression test.*

***Keywords:*** *Dexamethasone suppression test, Cushing's syndrome, Diagnostic test*

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Cushing's syndrome comprises of the symptoms and signs associated with prolonged exposure to inappropriately elevated level of glucocorticoids. The clinical features leading to the diagnosis of Cushing's syndrome include centripedal obesity, moon face, hirsutism, facial plethora, proximal muscle weakness, purplish striae, glucose intolerance, hypertension, menstrual irregularities, and neuropsychological disturbances such as depression, emotional irritability, sleep disturbances, and cognitive deficit. It can be classified into exogenous and endogenous Cushing's syndrome. Exogenous Cushing's syndrome is usually diagnosed by a history of exogenous steroid use. Endogenous Cushing's syndrome results from excessive production of glucocorticoid from adrenal cortex. The laboratory assessments of the patients suspected of having endogenous Cushing's syndrome are divided into 2 steps. The first step is to confirm the states of hypercortisolism, and the second one is to define the cause of Cushing's syndrome.

Due to the variable pattern of biochemical parameters and the nonspecificity of clinical manifestations, the diagnosis of Cushing's syndrome is often a challenge for clinicians. This is particularly true in states of mild hypercortisolism. Biochemical confirmation of endogenous Cushing's syndrome comprises of several different tests, but none has been proven fully capable of distinguishing all cases of Cushing's syndrome from normal or obese individuals. Tests that have been currently used as a first-line

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screening tests to establish the state of hypercortisolism are discussed below.

***Urine free cortisol (UFC)***

The 24-hour urinary cortisol gives an integrated index of the free cortisol circulating in the blood during this period of time. In contrast to the plasma cortisol levels, UFC is not affected by the factors that influence cortisol binding globulin levels[1]. UFC determination is thought to have a high sensitivity and specificity for the diagnosis of Cushing's syndrome. However, a falsely low UFC can occur in patient with renal impairment. In addition, UFC can be normal in patient with cyclic disease. False positive test for UFC has also been reported in patients with pseudo-Cushing conditions including depression, chronic alcoholism and patients taking carbamazepine and fenofibrate. Adequate urine collections are also required for the accuracy of the test thus making the test inconvenience for most of the patients.

***Late-night salivary cortisol***

Alteration of the circadian rhythm occurs in patients with Cushing's syndrome. Therefore, the absence of a late-night cortisol nadir is used for the diagnosis. Salivary cortisol measurement has been reported to yield 92-100% sensitivity and 93-100% specificity[2-7]. However, the circadian rhythm may be blunted in many patients with depressive illness, in shift worker and in the critically ill patient[8-10].

***Overnight 1 mg dexamethasone suppression test (ODST)***

This test is performed by giving 1 mg of dexamethasone orally between 23.00-24.00 hour, followed by measurement of fasting plasma cortisol between 08.00 and 09.00 hour in the following morning. The reported cutoff values in normal individuals are ranging from 1.8 to 7.3 μg/dl[11]. The original criterion for normal level of suppression was a plasma cortisol being less than 5 μg/dl. More recently this cutoff level has been reduced to less than 1.8 μg/dl, which greatly enhancing the sensitivity of the test, especially with mild hypercortisolism. However, lowering the cutoff level also increases the false positivity and decreases the specificity of the test[11-13]. Recent guideline recommends using the ODST as an initial test especially in patient with renal impairment and in patient with adrenal incidentaloma suspected of having Cushing's syndrome[14].

***The classic 2-day low dose dexamethasone suppression test (LDST)***

The patients have to take 0.5 mg of dexamethasone orally every 6 hours for 2 days. Urine is collected for UFC measurement or alternatively serum cortisol level is measured within 2-6 hours after the last dose. As similar to the ODST, the cutoff level for plasma cortisol has been currently reduced from 5 μg/dl to 1.8 μg/dl in order to increase the sensitivity of the test to detect patients with mild Cushing's syndrome[13]. The ODST and the LDST have similar sensitivity for diagnosis of Cushing's syndrome (90-100%) but the LDST has been reported to have a little more specificity than the overnight test (95-100% vs. 85-90%)[12,13,15-17]. However, the ODST may be preferable because of its simplicity, low cost, and it can be performed as an out-patient basis. Some authors stated that the traditional 2-day low dose test is no longer be recommended[1]. For any dexamethasone suppression test, false positive test may occur if the following conditions are existed; decreased dexamethasone absorption, increased concentration of cortisol binding globulin and drugs that accelerate hepatic clearance of dexamethasone via CYP3A4. The present study aims to evaluate the diagnostic accuracy of the ODST and the LDST, and to compare the efficacy between these two tests for diagnosis of Cushing's syndrome using cutoff value at 5 μg/dl (previous cutoff value) and 1.8 μg/dl (new cutoff value). The best cutoff values of the two tests were also determined.

## Material and Method

***Study design and population***

Data from patients who underwent endocrine evaluation for Cushing's syndrome at the Division of Endocrinology and Metabolism, Siriraj Hospital from 1971-2007 were collected and analyzed. A definite diagnosis of Cushing's syndrome was made on the basis of histopathological data obtained by surgery. Those who had no surgery, biochemical tests plus radiological studies compatible with adrenal tumor, pituitary tumor or ectopic ACTH production tumor were required.

The ODST was performed by giving 1 mg of dexamethasone at 23.00 hour and measure plasma cortisol at 8.00 hour in the next morning. Cutoff value at below 5 μg/dl has been used at Siriraj Hospital.

The LDST was performed by giving 0.5 mg of dexamethasone orally every six hours for two days. Serum cortisol at 8.00 hour in the next morning was

determined. Cutoff point of serum cortisol below 5 μg/dl has been used at Siriraj Hospital. The present study was approved by the ethical committee of Siriraj Hospital.

***Measurement of cortisol level***

Serum cortisol was measured by radioimmunoassay method (CIS bio international, France). Since the year 2000, both radioimmunoassay and chemiluminescent methods (Roche Diagnostic, Switzerland) have been used.

***Statistical analysis***

Data were recorded and analyzed by SPSS (SPSS Inc Chicago, IL USA). To test the agreement between the ODST and the LDST, Kappa statistical analysis was used[18]. Mean, standard deviations and Student's t-test were analyzed for normally distributed data. Sensitivity, specificity, positive predictive value and negative predictive value of the two tests were calculated and comparisons of the accuracy between cutoff value at below 5 μg/dl and at below 1.8 μg/dl were performed. Receiver operating characteristic (ROC) curves were created to determine the best cutoff value of the two tests.

## Results

A total of 77 patients, 11 men and 66 women were evaluated. Their mean age was 35 years (range, 17-74 years). Among 77 patients studied, 30 of them had adrenal adenoma, 26 had Cushing's disease, 6 had adrenal carcinoma, 1 had ectopic ACTH syndrome due to thymic carcinoid tumor, and 14 had pseudo-Cushing's syndrome. The ODST was performed in 61 patients and LDST was performed in 74 patients. As shown in Table 1, comparison between these two tests in 60 patients showed very good agreement by using Kappa statistical analysis (K = 0.84, p < 0.001).

By using the results of histopathological diagnosis, radiological studies, plasma ACTH and therapeutic outcomes as the gold standard of definitive diagnosis, the efficacy of the ODST and the LDST was calculated by using the traditional cutoff value at 5 μg/dl (Table 2). The sensitivity of the ODST

**Table 1.** Agreement between overnight dexamethasone suppression test (ODST) and the low dose dexamethasone suppression test (LDST)

| | LDST | | Total |
|---|---|---|---|
| | Non-suppressible | Suppressible | |
| ODST | | | |
| Non-suppressible | 47 | 2 | 49 |
| Suppressible | 1 | 10 | 11 |
| Total | 48 | 12 | 60 |

**Table 2.** Sensitivity, specificity and predictive values of the overnight dexamethasone suppression test (ODST) using cutoff value below 5 μg/dl

| Test | | Disease | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| ODST | Positive | 48 | 3 | 51 |
| | Negative | 0 | 11 | 11 |
| | Total | 48 | 14 | 62 |

Disease = definitive diagnosis of Cushing's syndrome

| Diagnostic parameters (%) | Value | 95% confidence interval |
|---|---|---|
| Sensitivity | 100.0 | 92.6-100.0 |
| Specificity | 78.6 | 52.4-92.4 |
| Positive predictive value | 94.1 | 84.1-98.0 |
| Negative predictive value | 100.0 | 74.1-100.0 |
| Accuracy | 95.2 | 86.7-98.3 |

**Table 3.** Sensitivity, specificity and predictive values of the low dose dexamethasone suppression test (LDST) using cutoff value below 5 μg/dl

| Test | | Disease | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| LDST | Positive | 62 | 1 | 63 |
| | Negative | 0 | 12 | 12 |
| | Total | 62 | 13 | 75 |

| Diagnostic parameters (%) | Value | 95% confidence interval |
|---|---|---|
| Sensitivity | 100.0 | 94.2-100.0 |
| Specificity | 92.3 | 66.7-98.6 |
| Positive predictive value | 98.4 | 91.5-99.7 |
| Negative predictive value | 100.0 | 75.7-100.0 |
| Accuracy | 98.7 | 92.8-99.8 |

**Table 4.** Sensitivity, specificity and predictive values of the ODST using cutoff value below 1.8 μg/dl

| Test | | Disease | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| ODST | Positive | 48 | 3 | 51 |
| | Negative | 0 | 11 | 11 |
| | Total | 48 | 14 | 62 |

| Diagnostic parameters (%) | Value | 95% confidence interval |
|---|---|---|
| Sensitivity | 100.0 | 92.6-100.0 |
| Specificity | 78.6 | 52.4-92.4 |
| Positive predictive value | 94.1 | 84.1-98.0 |
| Negative predictive value | 100.0 | 74.1-100.0 |
| Accuracy | 95.2 | 86.7-98.3 |

**Table 5.** Sensitivity, specificity and predictive values of the LDST using cutoff value below 1.8 μg/dl

| Test | | Disease | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| LDST | Positive | 62 | 2 | 64 |
| | Negative | 0 | 1 | 11 |
| | Total | 62 | 13 | 75 |

| Diagnostic parameters (%) | Value | 95% confidence interval |
|---|---|---|
| Sensitivity | 100.0 | 94.2-100.0 |
| Specificity | 84.6 | 57.8-95.7 |
| Positive predictive value | 96.9 | 89.3-99.1 |
| Negative predictive value | 100.0 | 74.1-100.0 |
| Accuracy | 97.3 | 90.8-99.3 |

**Table 6.** Comparison of the sensitivity, specificity, and predictive values of the original cutoff point (5 □g/dl) and the new cutoff point (1.8 □g/dl)

| Diagnostic parameters (%) | ODST | | LDST | |
|---|---|---|---|---|
| | <5 μg/dl | <1.8 μg/dl | <5 μg/dl | <1.8 μg/dl |
| Sensitivity | 100.0 | 100.0 | 100.0 | 100.0 |
| Specificity | 78.6 | 78.6 | 92.3 | 84.6 |
| Positive predictive value | 94.1 | 94.1 | 98.4 | 96.9 |
| Negative predictive value | 100.0 | 100.0 | 100.0 | 100.0 |
| Accuracy | 95.2 | 95.2 | 98.7 | 97.3 |

was 100%, and the specificity was 78.6%. Positive predictive value was 94.1% and negative predictive value was 100%. The LDST has the sensitivity of 100%, specificity of 92.3%, positive predictive value of 98.4% and negative predictive value of 100%.

By using the new cutoff value at below 1.8 μg/dl, the sensitivity and specificity of the two tests were calculated again. The sensitivity and specificity of the ODST were equal to those values using previous cutoff value at below 5 μg/dl. However, the specificity of the LDST was decreased from 92.3% to 84.6% and the positive predictive value was decreased from 98.4% to 96.9% when the new cutoff value at below 1.8 μg/dl was applied (Table 6). Receiver operating characteristic (ROC) curves were used to determine the best cutoff value of the two tests. Cortisol was measured by two methods in this data (Radioimmunoassay and chemiluminescent), therefore patients were separately analyzed according to the method of measurement that has been used. Due to the small numbers of patients with pseudo-Cushing's syndrome using the Radioimmunoassay method, only the cortisol cutoff value of the chemiluminescent technique was determined. Two patients with false positive result of ODST were excluded from the calculation of ROC curve because their tests were done while the patients were acutely ill. The first patient had the test done during heart failure and the second patient was examined during an admission with fracture neck of the femur. ROC curves showed that the best cutoff value of ODST was ≥ 5.3 μg/dl. Area under the ROC is 1.0 (95% CI 0.89-1.0) (Fig. 1). And for LDST test, the best cutoff value was ≥ 5 μg/dl. Area under the ROC is 1.0 (95% CI 0.87-1.0) (Fig. 2)

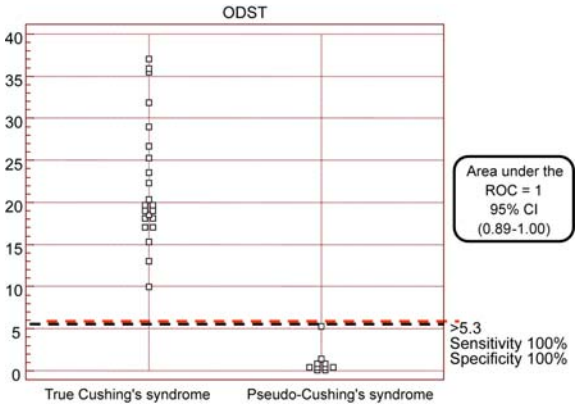


**Fig. 1** The best cutoff value of overnight dexamethasone suppression test (ODST) using the ROC curve

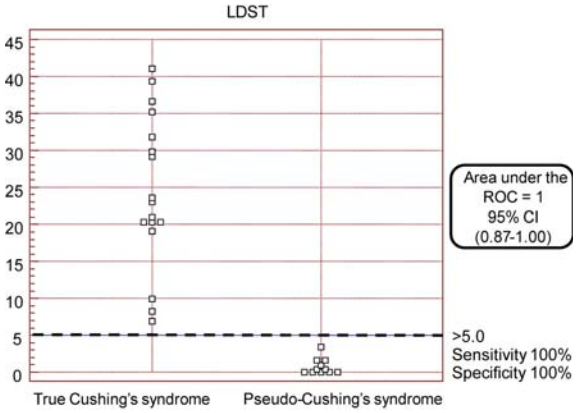


**Fig. 2** The best cutoff value of low dose dexamethasone suppression test (LDST) using the ROC curve

## Discussion

Even though the diagnostic test for Cushing's syndrome has a long history, but the choice of optimal screening procedure and the best cutoff level remains controversial[19]. Recent meta-analysis showed that both ODST and LDST had good diagnostic accuracy although LDST was slightly less accurate than ODST[20]. Previous survey of laboratories in the United Kingdom indicated that the majority of pathologists recommended the ODST as a screening procedure for patients suspected of having Cushing's syndrome[12,21]. From our data, ODST is an efficient method for the screening of patients suspected of having Cushing's syndrome and has very good agreement with the LDST. It has advantages over the LDST because of its ease of execution, lower cost and it can be performed as an out-patient basis. Therefore, ODST may be more preferable than LDST as the diagnostic test for patients suspected of Cushing's syndrome. However, because of limited sample size in this study, especially the small numbers of normal subjects, specificity of the test may be inaccurately estimated. Thus, further studies with larger sample size are needed before a recommendation of using ODST to replace LDST could be made.

Reported cutoff values for the suppression of serum cortisol in other studies using contemporary immunoassay techniques range from 3.6-7.2 μg/dl[16]. A widely cited cutoff value is a serum cortisol being less than 5 μg/dl[15]. A consensus opinion by pathologists in the United Kingdom, which was based on available clinical data, stated that dexamethasone induced suppression of serum cortisol to less than 1.8 μg/dl effectively excludes Cushing's syndrome[12]. The application of this stringent cutoff to safely exclude Cushing's syndrome was also endorsed at an international workshop on the diagnosis, complications, and treatment of Cushing's syndrome[13]. A much lower cutoff value has been demonstrated to improve the sensitivity of the test. Moreover, as the cutoff value is lowered to increase the sensitivity, the specificity significantly decreased; thereby decreasing the overall diagnostic utility of the test[22]. Our study has shown that lowering the cutoff values did not change the sensitivity of both dexamethasone suppression tests, but it decreased the specificity of the LDST and slightly decreased the diagnostic accuracy of the test. For the ODST, lowering the cutoff value resulted in the same sensitivity, specificity and accuracy as compared with the previous cutoff value. From our data, the sensitivity of both tests are 100% since using the cutoff value at 5 μg/dl , as a result, the benefit of lowering the cutoff value is not seen. The reason that might explain why the sensitivity is 100% in this study is because our center is a referral center; therefore, most of the cases had full-blown Cushing's syndrome. In addition, the problematic case for diagnosis (a patient with mild Cushing's syndrome or cyclical disease) was not found in our study. The ROC curves show that the best cutoff value is 5.3 μg/dl with ODST and 5 μg/dl with LDST. As a result, we support the use of traditional cutoff value at 5 μg/dl. Lowering the cutoff will lead to unnecessary investigations and increased cost. Further study about the cost effectiveness is necessary. Moreover, there is no evidence to support that the final outcome in these mild cases is better with treatments. It might be more cost effectiveness to use higher cutoff value at 5 μg/dl and consider

further investigation in individual with normal test in whom the pretest probability is high. However, individual with normal test result and Cushing's syndrome is unlikely, re-evaluation in 6 months should be considered if signs and symptoms progress.

In conclusion, the ODST is an efficient method and is comparable to the LDST for diagnosis of patients suspected of having Cushing's syndrome. However, the extent of laboratory investigations will depend on index of clinical suspicion. Some patients with mild hypercortisolism may require several tests and long term follow up to establish the final diagnosis. The attempt to lowering cutoff value does not improve the efficacy of the dexamethasone suppression test.

***Declaration of interest***

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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## *การศึกษาเปรียบเทียบประสิทธิภาพของการทดสอบ 1 mg overnight dexamethasone suppression test (ODST) และ 2-day low dose dexamethasone suppression test (LDST) ในการวินิจฉัยกลุ่มอาการ Cushing*

***นันทกร ทองแตง, สาธิต วรรณแสง***

***วัตถุประสงค์:*** *เพื่อศึกษาความไวและความจำเพาะของการทดสอบ 1 mg overnight dexamethasone suppression test (ODST) และ 2-day low dose dexamethasone suppression test (LDST) ในการวินิจฉัยกลุ่มอาการ Cushing และเพื่อเปรียบเทียบประสิทธิภาพของการทดสอบทั้งสองนี้โดยใช้จุดตัดที่ 5 ไมโครกรัม/ดล. และ 1.8 ไมโครกรัม/ดล.*

***วัสดุและวิธีการ:*** *ได้ทำการศึกษาย้อนหลังตั้งแต่ปีพ.ศ. 2514 ถึง พ.ศ.2550 ในผู้ป่วยโรงพยาบาลศิริราช ที่มีลักษณะทางคลินิกเข้าได้กับกลุ่มอาการ Cushing โดยวิเคราะห์ ความไวและความจำเพาะของการทดสอบ ศึกษาความคล้องจองกันของการทดสอบ 2 ชนิดโดยใช้การวิเคราะห์ทางสถิติของ Kappa และใช้ ROC curve ในการหาค่าจุดตัดที่ดีที่สุดของการทดสอบ*

***ผลการศึกษา:*** *ODST เป็นการทดสอบมีความคล้องจองกันเป็นอย่างดีกับการทดสอบ LDST และมีประสิทธิภาพใกล้เคียงกัน การลดค่าจุดตัดจาก 5 ไมโครกรัม/ดล. เป็น 1.8 ไมโครกรัม/ดล. ไม่ได้เพิ่มความแม่นยำของ ODST และลดความจำเพาะของ LDST ค่าจุดตัดที่ดีที่สุดของ ODST คือ 5.3 ไมโครกรัม/ดล. และ ค่าจุดตัดที่ดีที่สุดของ LDST คือ 5 ไมโครกรัม/ดล.*

***สรุป:*** *ODST เป็นการทดสอบที่มีประสิทธิภาพในการวินิจฉัยผู้ป่วยกลุ่มอาการ Cushing การลดค่าจุดตัด ไม่ได้เพิ่มประสิทธิภาพของการทดสอบ ODST และ LDST*