Original Article

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New Cut-off Value for Low-Dose Acth Stimulation Test in the Diagnosis of Adrenal Insufficiency

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Abstract

Aim: Although the peak cortisol response in the low-dose test does not reach the generally accepted value of 18 µg/dL, there are many patients whose adrenal insufficiency diagnosis was ruled out by getting an adequate response with the standard-dose test. Thus, the standard-dose adrenocorticotropic hormone (ACTH) stimulation test is unnecessarily applied to many patients. This study aims to compare low- and standard-dose ACTH stimulation tests in diagnosing adrenal insufficiency and to determine a new optimal threshold level for low-dose ACTH stimulation tests to avoid unnecessary standard-dose ACTH tests.

Methods: In this single-center cross-sectional study, patients with suspected adrenal insufficiency who underwent low-dose (1 mcg) and standard-dose (250 mcg) ACTH stimulation tests were evaluated. Cases were separated into two groups: (I) inadequate cortisol response (<18 mcg/dL) to low-dose test and adequate response (≥18 mcg/dL) to the standard dose, (II) adrenal insufficiency by inadequate responses to both tests. In addition, the cortisol responses to the stimulation tests of the group not diagnosed with adrenal insufficiency and those diagnosed with adrenal insufficiency were compared.

Results: Comparing 115 cases' peak cortisol levels, we found that the values of standard-dose tests were statistically significantly higher than those of low-dose tests ($p<0.001$). According to the receiver operating characteristic analysis we performed to determine a new cut-off level to eliminate adrenal insufficiency in the low-dose test, when the cut-off value was set as 16 µg/dL; 100% sensitivity, 35.64% specificities, 17.72% positive predictive value, and 100% negative predictive value were obtained. It has been shown that this new cut-off value would eliminate the diagnosis of adrenal insufficiency without performing the standard dose test in 36 cases.

Conclusion: The need for standard-dose testing can be reduced by lowering the cut-off point of the low-dose test from the accepted adequate cortisol level of 18 mcg/dL to 16 mcg/dL.

Keywords: Adrenal insufficiency, low-dose ACTH stimulation test, standard-dose ACTH stimulation test

Introduction

Adrenal insufficiency is a life-threatening condition that may cause different clinical presentations. If the early morning serum cortisol level is below 3 mcg/dL, adrenal insufficiency should be considered a definitive diagnosis (1). To evaluate hypothalamic-pituitary-adrenal (HPA) axis functions in cases with 3-18 mcg/dL basal cortisol levels and clinically suspected adrenal insufficiency, the insulin-induced hypoglycemia test (IHT) is recommended as the gold standard test (2). However, IHT is contraindicated in patients with arrhythmia, ischemic heart disease, or epilepsy because of the risks of hypoglycemia and should be applied with caution in elderly patients. It is also a seriously irritating test for patients, requiring hospitalization and close medical observation for testing. Thus, adrenocorticotropic hormone (ACTH) stimulation tests are the most commonly used method to evaluate the HPA axis in daily practice (3,4).

In ACTH stimulation tests, tetracosactide, a synthetic analog of ACTH (also known as cosyntropin- ACTH 1-24), is used. Performing the test with 250 mcg is called the standard dose, and performing the test with 1 mcg is called the low-dose ACTH stimulation test. Cortisol levels are measured at the 30th and 60th minutes after the administration of synthetic ACTH (cosyntropin), and the

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highest value is used to evaluate the response to the stimulation test (5). However, some authors consider the dose used in standard-dose ACTH stimulation to be above the physiological level. Therefore, a low-dose test using 1 mcg of cosyntropin has emerged to prevent supraphysiological stimulation of the adrenal cortex (6).

Although the low-dose test has been reported to be more correlated with IHT than the standard-dose test in patients after pituitary surgery (7), it was found that when specific threshold values are applied to low- and standard-dose ACTH stimulation tests, they are similar in sensitivity and accuracy (8).

A low-dose test is performed first in cases of suspected adrenal insufficiency. If the peak cortisol level is between 3 and 18 mcg/dL, a standard dose test is performed because adrenal insufficiency cannot be ruled out. If the cortisol level is above 18 µg/dL at the 30th or 60th minute, adrenal insufficiency can be ruled out (9). Values below 18 µg/dL should be considered adrenal insufficiency. However, although the peak cortisol response in the low-dose test does not reach the generally accepted value of 18 µg/dL, there are many patients whose adrenal insufficiency diagnosis was ruled out by getting an adequate response with the standard dose test. Thus, the standard-dose ACTH stimulation test is unnecessarily applied to many patients. Therefore, this study aimed to compare low- and standard-dose ACTH stimulation tests in diagnosing adrenal insufficiency and to determine a new optimal threshold level for low-dose ACTH stimulation tests to avoid unnecessary standard-dose ACTH tests.

Methods

Compliance with Ethical Standards

The University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital Local Ethics Committee reviewed and approved this study protocol on February 25, 2020 (approval number: 1458). Participants were informed that the data would be used for scientific purposes only.

Design and Study Population

This study was conducted as a retrospective, cross-sectional, single-center study. Patients who applied to the endocrinology department between January 2013 and December 2019 were screened for the study. Patients with clinical suspicion of adrenal insufficiency who underwent low- and standard-dose ACTH stimulation tests were included because the basal cortisol level was approximately 3-18 µg/dL in the examinations. The age range of the included patients was 18-80, and the study was conducted with 115 patients. Patients with congestive heart failure, uncontrolled diabetes mellitus, hypertension, chronic kidney injury, thyroid dysfunction, and drug use that would affect the metabolism of the synthetic ACTH analog cosyntropin used in the test were excluded from the study. Moreover, patients who had used topical or systemic steroids in the last 48 hours before the test were also excluded from the study.

Patients were separated into two groups according to the response to the low- and standard-dose ACTH stimulation tests: (1) inadequate response to the 1 mcg ACTH stimulation test (<18 mcg/dL at the 30th or 60th minutes) but adequate response to the 250 mcg ACTH stimulation test (≥18 mcg/dL at the 30th or 60th minutes), (2) inadequate response to both tests, thus diagnosed with adrenal insufficiency. Between Group 1 without adrenal insufficiency and Group 2 with primary or secondary adrenal insufficiency, the responses to the low- and standard-dose ACTH stimulation tests at the 30th and 60th minutes and the increase in basal serum cortisol values were compared. Receiver operating characteristic (ROC) analysis was applied to determine the optimal threshold value and diagnostic accuracy of the low-dose ACTH stimulation test in diagnosing adrenal insufficiency.

A 250 mcg preparation of cosyntropin (tetracosactrin 1-24, Synacten®), a synthetic analog widely used for ACTH stimulation tests, was used intravenously. A solution was prepared with 250 mcg of Synacthen® preparation diluted with 50 mL of normal saline for low-dose ACTH stimulation. This solution was used under the condition that it be stored for a maximum of three months at +4 degrees. When performing low-dose ACTH testing, 0.2 mL was taken from this solution, diluted to 10 mL with 0.9% NaCl, and then applied to the patient.

Blood samples were taken for basal cortisol at 8:00 a.m. After sampling, 1 mcg of the previously prepared solution was given as a bolus injection. Then, at the 30th and 60th minutes, blood samples were taken for plasma cortisol levels. At least three days later, the test was repeated in the same protocol, using 250 mcg of synthetic ACTH instead of 1 mcg.

Cortisol measurements were made with the COBAS 602 device between 2013 and 2017 and the Beckman DXI 800-3 device between 2017 and 2019. For measurement, the electroluminescence method was used. A plasma cortisol level of 18 mcg/dL or above during the test at the 30th and/or 60th minutes is considered a normal adrenal function indicator.

Statistical Analysis

Statistical analyses were performed using the SPSS version 17.0 program. The normality of the variables was examined using histograms and the Kolmogorov-Smirnov test. Mean, standard deviation, and median values were used in descriptive analyses. The Mann-Whitney U test

was used to evaluate non-normally distributed (non-parametric) variables between two groups. A ROC analysis was performed to determine a new cut-off value for detecting adrenal insufficiency. Cases with a p-value below 0.05 were considered statistically significant.

Results

The study was executed with 115 patients, 40 males and 75 females. The mean age of the cases was 42.21±13.96 years, and 14 patients were diagnosed with adrenal insufficiency, seven of whom had primary adrenal insufficiency and seven had secondary adrenal insufficiency.

Responses to the low- and standard-dose ACTH stimulation of the patients are shown in Figures 1A and 1B.

When the cortisol results were compared to the minutes, the mean of the 30th and 60th-minute levels of the patients who underwent the standard dose test was statistically significantly higher than the low dose test (Table 1).

After the administration of cosyntropin, the percentage increase in cortisol with the 250 mcg test (151.86±148.90%) was significantly higher than the increasing percentage of the 1 mcg test (97.00±93.35%) (p<0.001) (Figure 2).

In the low-dose stimulation test, ROC analysis was performed over the 30th-minute values to determine a new cut-off value to rule out the suspicion of adrenal insufficiency. When the optimal cut-off value was taken at 16 mcg/dL at the 30th minute, 100% sensitivity, 35.64% specificity, 17.72% positive predictive value (PPV), and 100.00% negative predictive value (NPV) were obtained (Figure 3). When the 30th-minute cut-off value is taken at 16 mcg/dL in the low-dose test, 79 patients are referred to the second-line test, and adrenal insufficiency is detected in 14 of them. Therefore, this new cut-off value concluded that no standard dose test would be needed for 36 patients.

The ROC analysis found a cut-off value of 14.80 mcg/dL for primary adrenal insufficiency and 16 mcg/dL for secondary adrenal insufficiency. According to these values, 100% sensitivity, 67.33% specificity, 17.50% PPV, and 100% NPV were obtained when the threshold point was taken as 14.80 mcg/dL for primary adrenal insufficiency. On the other hand, when the threshold value of 16 mcg/dL was taken for secondary adrenal insufficiency, 100% sensitivity, 35.64% specificity,

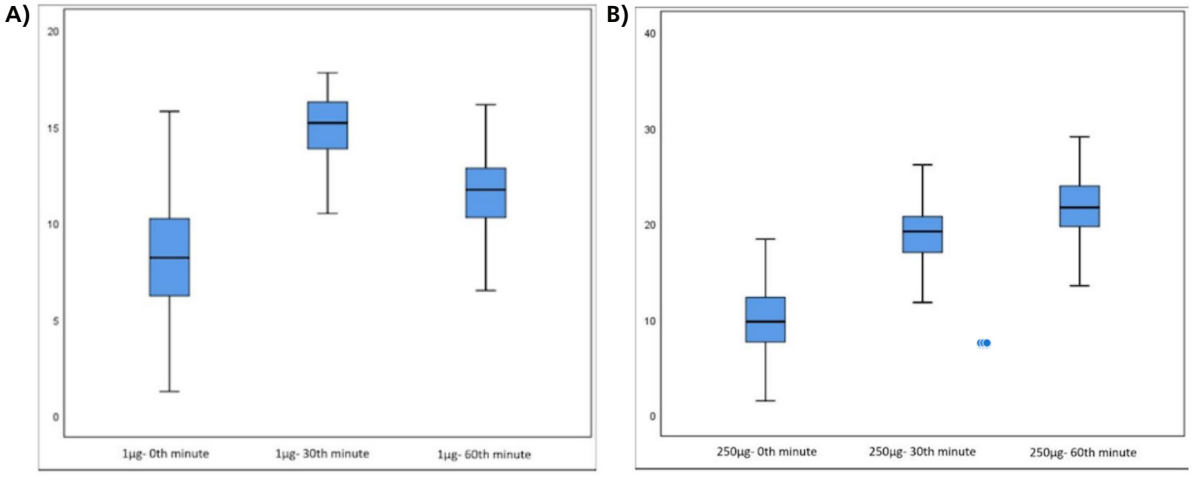

Figure 1. A) Responses to the low-dose ACTH stimulation test of the patients. **B)** Responses to the standard-dose ACTH stimulation test of the patients
ACTH: Adrenocorticotropic hormone

Table 1. Comparison of the results of low-dose and standard-dose ACTH stimulation tests by minutes

	Basal (0 min)		30 min		60 min	
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median
1 mcg ACTH	8.54±2.86	8.20	14.77±2.27	15.20	11.54±2.49	11.73
250 mcg ACTH	10.07±3.73	9.76	18.87±3.67	19.20	21.34±4.37	21.70
p-value*	0.14		0.01		<0.001	

*Mann-Whitney U test
Min: Minute, SD: Standard deviation, ACTH: Adrenocorticotropic hormone

9.72% PPV, and 100% NPV were obtained (Figures 4A and 4B).

For the detection of primary adrenal insufficiency, when the 30th-minute cut-off value is 14.80 mcg/dL in the low-dose test, 40 of 108 patients are referred to the second-line test, and primary adrenal insufficiency is detected in 7 of them. With this new cut-off value, 68 patients were not referred to the second step. For the diagnosis of secondary adrenal insufficiency, when the 30th-minute cut-off value is 16 mcg/dL in the low-dose test, 72 of 108 patients are referred to the second-line test. Secondary adrenal insufficiency was detected in seven of them. With this new cut-off value, 36 patients were not referred to second-line testing.

No statistically significant results were obtained in the ROC analysis to determine the threshold value using the percentage increase compared to the baseline to detect adrenal insufficiency. In addition, while there was a low and statistically significant correlation between the low and standard dose test 30th-minute values, no statistically significant correlation was observed between the 60th-minute values (Figure 5A, Figure 5B).

When the 30th and 60th-minute values were compared between low- and standard-dose tests, the 30th and 60th-

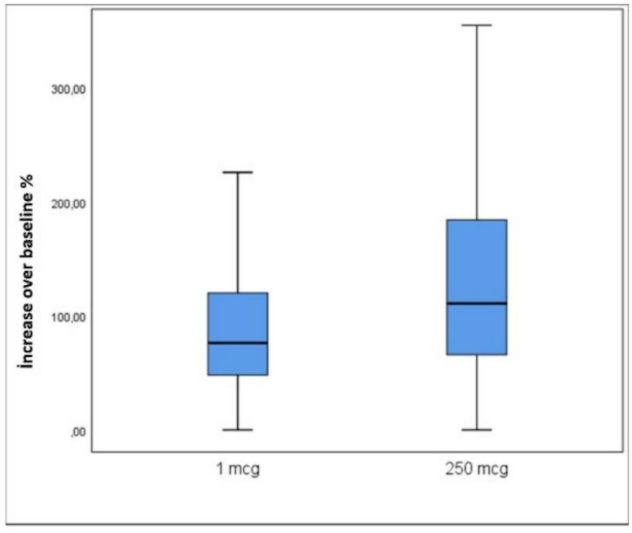

Figure 2. Percentages of increase in low-dose and standard-dose ACTH stimulation tests compared to basal
ACTH: Adrenocorticotropic hormone

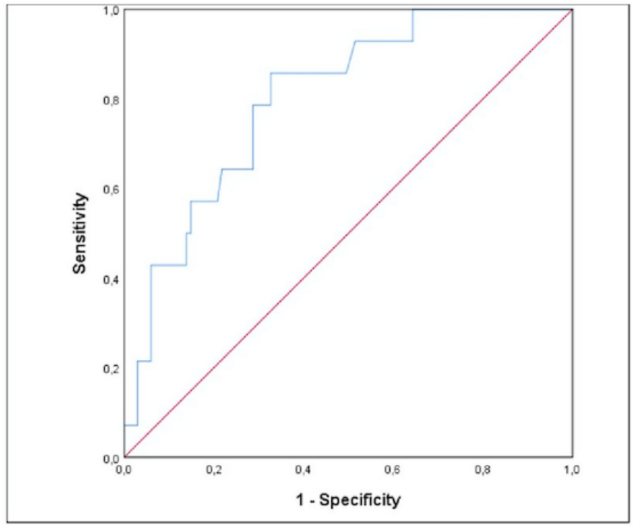

Figure 3. ROC analysis for the determination of low-dose ACTH test 30. minute value for adrenal insufficiency
ROC: Receiver operating characteristic, ACTH: Adrenocorticotropic hormone

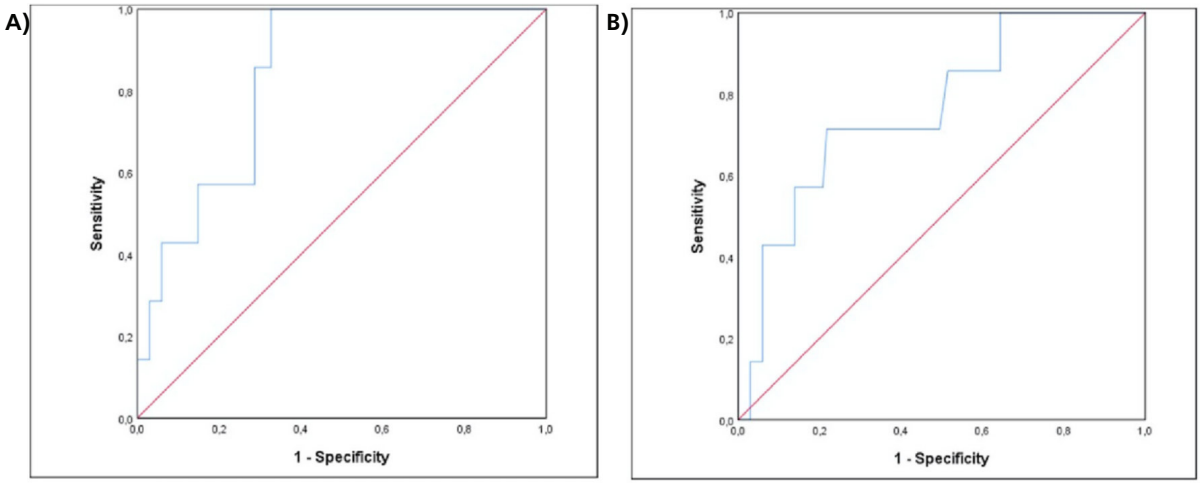

Figure 4. A) ROC analysis for the estimation of low-dose ACTH test 30th-minute value for primary adrenal insufficiency. **B)** ROC analysis for the estimation of low-dose ACTH test 30th-minute value for secondary adrenal insufficiency
ROC: Receiver operating characteristic, ACTH: Adrenocorticotropic hormone

minute values of the standard-dose test were significantly higher than those of the low-dose test (Table 2).

Discussion

This study provides information on the effectiveness of the low-dose ACTH stimulation test in screening and diagnosing adrenal insufficiency. In this study, it has been shown that when 16 mcg/dL is taken instead of the generally accepted 18 mcg/dL threshold value in the low-dose test, a high rate of 31.3% (36/115) of the patients exclude the diagnosis without the need for a standard dose test. Thus, it has been concluded that fewer patients will be exposed to supraphysiological doses of ACTH and that the test can be used more cost-effectively.

In evaluating the HPA axis, if the basal cortisol value is below 3 mcg/dL, intense HPA deficiency is considered. In comparison, if it is above 18 mcg/dL, the HPA axis is considered normal. No clear interpretation can be made between these values. In cases of clinical suspicion and necessity, the HPA axis is investigated with dynamic tests. The gold standard test accepted today in dynamic tests is IHT (3,7-11). The literature has different threshold values regarding interpreting cortisol values obtained by IHT. Also, this test cannot be applied in the presence of ischemic heart disease, epilepsy, and cerebrovascular disease. When applied, it requires close medical follow-up (12). Although IHT is the gold standard test for HPA axis evaluation, the ACTH stimulation test is preferred due to its low side effects (10).

Exogenously administered synthetic ACTH has been used to assess the HPA axis for many years. Wood et al. (13) measured plasma cortisol levels by administering intramuscular synthetic ACTH in the groups with normal adrenal functions, inadequate functions, and suspected insufficiency and showed that adrenal function could be evaluated at 30. min. Although some authors have stated that the standard dose test has more correlated results with IHT, it is well above the physiological dose of 250 mcg, and a study reported that it stimulates the adrenal gland 25 times more than the normal physiological response (5). Moreover, it has been shown that the 1 mcg test is more sensitive to secondary adrenal insufficiency (14). If the peak cortisol level is above 18 mcg/dL after a 1 mcg test, the patient is considered to have no adrenal insufficiency (15). Schultz et al. (16) showed that serum cortisol levels increase to 18 mcg/dL under stress in healthy individuals.

There is no clarity in the literature about the optimal threshold values for cortisol in response to dynamic testing. A study suggested values ≥19 mcg/dL as an

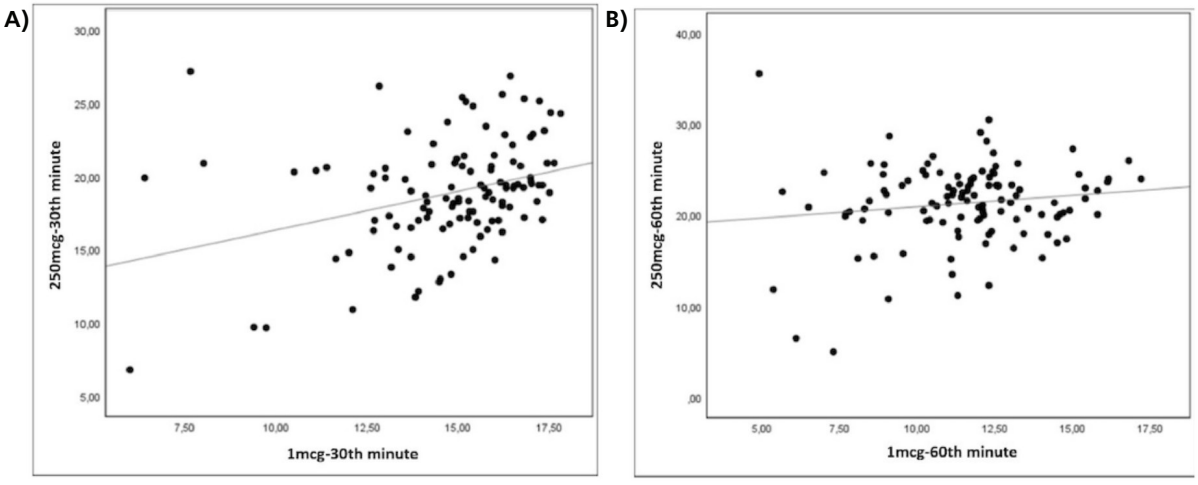

Figure 5. A) Correlation between low dose ACTH test 30th-minute and standard dose ACTH test 30th-minute values. **B)** Correlation between low dose ACTH test 60th-minute and standard dose ACTH test 60th-minute values
ACTH: Adrenocorticotropic hormone

Table 2. Comparison of peak cortisol values in low-dose and standard-dose ACTH stimulation test

	1 µg		250 µg		p-value*
	Mean±SD	Median	Mean±SD	Median	
30th min.	14.77±2.27	15.20	18.87±3.67	19.20	**<0.001**
60th min.	11.54±2.49	11.73	21.34±4.37	21.70	**<0.001**

*Mann-Whitney U test
Min: Minute, SD: Standard deviation, ACTH: Adrenocorticotropic hormone

adequate response to the low-dose test (17). In another study, 16 patients were evaluated with IHT and a 1 mcg test. When 18 mcg/dL was accepted as the threshold value for both tests, it was found that all patients responded adequately to the IHT and 1 mcg tests (18). A meta-analysis has commented that the lowest cortisol response in response to the 1 mcg test in healthy individuals may be lower than 12.5 mcg/dL. The same meta-analysis compared the 250 mcg and 1 mcg tests, and the optimal timing for measuring cortisol response was investigated. No significant difference was found in any study's diagnostic distinction between the 30th and 60th minutes and peak cortisol values. Again, the same meta-analysis compared the diagnostic performances of 1 mcg and 250 mcg ACTH stimulation tests. It was shown that the 1 mcg test performed better in diagnosis due to better sensitivity (19).

A study concluded that the cortisol threshold value sufficient for the low-dose test was 12.5 mcg/dL, based on a comparison of 1 mcg ACTH, 250 mcg ACTH, and glucagon stimulation test (GST) in 55 healthy volunteers aged 25-69 years. Peak cortisol level was reached at 30 min with the 1 mcg ACTH test, 90 min after the 250 mcg ACTH test, and 180 min after GST. The mean peak cortisol response to the 250 mcg ACTH test was significantly higher than that to 1 mcg ACTH and GST. While a positive correlation was found between mean peak cortisol responses in 1 mcg and 250 mcg ACTH stimulation tests, no significant correlation was found between GST and ACTH stimulation tests (20).

In our study, the lowest peak cortisol response of the cut-off value at the 30th minute in the low-dose ACTH stimulation test was 16 µg/dL. In the low-dose ACTH stimulation test for primary and secondary adrenal insufficiency, the lowest peak cortisol responses of the cut-off value at the 30th minute were 14.80 mcg/dL and 16 mcg/dL, respectively. In a meta-analysis, peak cortisol responses lower than 16 mcg/dL and higher than 30 mcg/dL in response to the standard dose test were high predictors of HPA axis insufficiency and adequacy, respectively. For the low-dose test, a peak cortisol response lower than 16 mcg/dL and higher than 22 mcg/dL, respectively, has been suggested as the best predictor for evaluating HPA axis insufficiency and adequacy (19). Abdu et al. (21) showed that the 21.75 mcg/dL cut-off value is safer than 18.12 mcg/dL for low- and standard-dose testing in clinical decision-making and treatment practice. If the response to the low-dose test is more excellent than 21.75 mcg/dL, it has 100% sensitivity, showing that the HPA axis is intact; it has been shown that less sensitivity and 3% false positive results are obtained at the standard dose compared to the low dose (21).

There are conflicting studies that show that the peak response to the 1 mcg test is altered in obese individuals. In a recent study, it was reported that the peak response to the 1 mcg test in obese individuals is lower than that of healthy controls (22). In our retrospective study, since not all individuals had body mass index (BMI) values, it was not possible to evaluate whether there was a change in the peak cortisol response in the 1 mcg test according to BMI.

A study analyzed 103 patients who underwent 1 mcg ACTH stimulation tests to determine the threshold value. Primary adrenal insufficiency was found in two of the four patients, and secondary adrenal insufficiency in two. When the standard threshold value was 18.12 mcg/dL, they reported 100% sensitivity, 67.3% specificity, and a high rate of false positive results. When 14.53 mcg/dL was taken as the threshold value, the sensitivity was 100% and the specificity was 93.9%. When 14.53 mcg/dL was taken as the threshold value in the low-dose test, it was observed that the rate of false positivity decreased significantly. Still, the sensitivity remained high (23). Our study obtained 100% sensitivity, 35.64% specificity, 17.72% PPV, and 100.00% NPV when the cut-off value for cortisol response to the low-dose test was more than 16 µg/dL. When the threshold value was 18 µg/dL, 100% sensitivity, 0% specificity, 12.17% PPV, and 0% NPV were obtained.

The study of Dekkers et al. (24) with 207 patients compared the cortisol response to the 1 mcg and 250 mcg ACTH stimulation tests in patients with suspected adrenal insufficiency. The mean difference between cortisol responses in both ACTH tests was 0.94 mcg/dL; a statistically significantly higher response was obtained in the 250 mcg ACTH test. In the study, the cut-off value of cortisol was taken as 19.93 mcg/dL; the diagnostic performances of both tests were found to be similar; however, it was observed that statistically significant average test results were obtained in the 250 mcg test. Individually, it has been observed that the difference in the cortisol response in both tests may be statistically significant. The response in the 250 mcg test may not always be higher than that in the 1 mcg test. Cortisol responses given at the 30th minute were correlated with each other in both tests (24).

The 1 mcg test is the lowest dose that gives the maximal cortisol response, but which dose should be applied in the initial evaluation is still controversial. The supraphysiological dose of 250 mcg is less sensitive in evaluating mild secondary adrenal insufficiency. Tordjman et al. (25) suggested that the 1 mcg test is more sensitive to secondary adrenal insufficiency and can replace the 250 mcg ACTH test. In our study, while there was a low

degree of similarity in the 30th-minute response to low- and standard-dose tests, there was no similarity between the 60th-minute values.

In another study, although the healthy volunteers underwent low- and standard-dose tests and had different baseline cortisol levels, the mean cortisol response at the 30th minute was similar. At the 60th and 90th minutes of the 1 mcg test, the mean cortisol response was significantly lower than the standard dose test. Again, the same study observed that the cortisol response to the ACTH test at the 30th minute did not change with basal cortisol values or at any time of the day. It was observed that the peak cortisol response reached the 30th minute in most of the 1 mcg ACTH stimulation tests (26). In our study, the average response to the low-dose test at the 30th minute was higher than that at the 60th minute; in the standard-dose test, the cortisol response at the 60th minute was higher than that at the 30th minute.

Studies have shown that an increase of 7 μg/dL or two times the value at the 0th minute, is expected with the ACTH stimulation test (27). In a study conducted with 21 healthy individuals, it was assumed that the increase in cortisol value was unsuitable for estimating adrenal insufficiency (28). In our study, the percentages of increases from baseline were compared between groups. Accordingly, the percentage of the increase of 250 mcg (151.86±148.90%) was significantly higher than the percentage of the increase of 1 mcg (97.00±93.35). However, it was concluded that the increase in cortisol levels is unsuitable for predicting adrenal insufficiency.

Study Limitations

The study was designed retrospectively. The BMI of individuals could not be evaluated because of a lack of data, although some studies show that obesity can alter the cortisol response to the 1 mcg ACTH stimulation test. Another limitation is that the stimulation tests were terminated after the 60th minute. In fact, in the generally accepted ACTH stimulation tests, it is considered sufficient to look at the 30th and 60th minutes to evaluate the cortisol response, which is routine practice. However, in the study of Karaca et al. (20), a peak cortisol response was reached at the 120th minute of standard dose test application in 55 healthy volunteers. In their study, if the standard-dose ACTH stimulation test were terminated after 60 minutes, 11% of the individuals would have been interpreted as having adrenal insufficiency. It was concluded that even if it was terminated after the 30th minute, the false positive rate would increase to 20% (20). For this reason, if 20 mcg/dL is not reached for 90 min in the standard dose test, an approach recommends extending the test time to 120 min if there are no clinical signs of adrenal insufficiency.

Conclusion

In this study, a 1 mcg ACTH stimulation test was found to be helpful in the diagnosis of adrenal insufficiency. In the 1 mcg ACTH stimulation test, when 16 μg/dL was taken instead of the generally accepted 18 μg/dL cut-off value at 30 min, it was shown that 31.3% of the patients did not need to apply the standard dose test. This will benefit both in terms of cost-effectiveness and by reducing exposure to supraphysiological ACTH doses. In this regard, future studies with more extensive series should determine the best cut-off values.

Ethics

Ethics Committee Approval: The University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital Local Ethics Committee reviewed and approved this study protocol on February 25, 2020 (approval number: 1458).

Informed Consent: Participants were informed that the data would be used for scientific purposes only.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K.T., M.M.C., Concept: M.M.C., Y.A., Design: M.M.C., Y.A., Data Collection or Processing: B.K.T., Analysis or Interpretation: B.K.T., M.M.C., Y.A., Literature Search: B.K.T., M.M.C., Writing: B.K.T., M.M.C., Y.A.

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