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Original Article

Clinical Characteristics, Indications, and Outcomes of the Low-Dose ACTH Stimulation Test in a Pediatric Endocrinology Center

Berna EROĞLU FİLİBELİ[1], Gülümay VURAL TOPAKTAŞ[1], Bumin N. DÜNDAR[2]

[1]University of Health Sciences Türkiye, İzmir City Hospital, Clinic of Pediatric Endocrinology, İzmir, Türkiye
[2]İzmir Katip Çelebi University Faculty of Medicine, Department of Pediatric Endocrinology, İzmir, Türkiye

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ABSTRACT

Objective: The low-dose adrenocorticotropic hormone stimulation test (LDAT) is widely used to evaluate the hypothalamic-pituitary-adrenal axis in pediatric endocrinology, particularly in suspected central adrenal insufficiency. This study aimed to retrospectively evaluate the clinical characteristics, indications, and outcomes of LDAT in a tertiary pediatric endocrinology center.

Methods: This retrospective study included 120 pediatric patients who underwent LDAT. A peak serum cortisol level ≥18 µg/dL at 30 minutes was considered an adequate adrenal response. Demographic, clinical, and laboratory data were analyzed. Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of baseline cortisol levels.

Results: A total of 120 patients (61 females, 59 males) were included. The median age was 11.5 years (interquartile range: 5.6); 55.8% were pubertal. The most common indication for testing was suspected central hypothyroidism (30.8%), followed by short stature or growth hormone deficiency (27.5%), and intracranial lesions or malformations (21.7%). An insufficient cortisol response was observed in 24.2% of patients. No significant differences were found between patients with adequate and insufficient responses regarding age, sex, anthropometric characteristics, or test indications ($p>0.05$). Baseline cortisol levels were significantly higher in patients with adequate responses (9.02±3.73 vs. 5.33±3.94 µg/dL, $p<0.001$). ROC analysis showed moderate diagnostic performance (area under the curve =0.75): a cut-off value of 5.05 µg/dL yielded 86.8% sensitivity and 55.2% specificity. None of the patients with baseline cortisol >13 µg/dL had an insufficient response.

Conclusion: LDAT remains a practical tool for evaluating adrenal function in children. Baseline cortisol may help identify low-risk patients, but dynamic testing remains essential for definitive diagnosis.

Keywords: Low-dose ACTH test, central adrenal insufficiency, pediatric endocrinology, cortisol, HPA axis

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Corresponding Author:
Berna EROĞLU FİLİBELİ
University of Health Sciences Türkiye, İzmir City Hospital, Clinic of Pediatric Endocrinology, İzmir, Türkiye
bernafilibeli@gmail.com
ORCID ID: 0000-0002-2696-0195

INTRODUCTION

Accurate assessment of the hypothalamic-pituitary-adrenal (HPA) axis is essential for the diagnosis of central adrenal insufficiency (CAI). Identifying patients with impaired adrenal function is crucial to prevent life-threatening complications, such as adrenal crisis, while avoiding overdiagnosis in individuals with a normally functioning axis is equally important. The insulin tolerance test (ITT) has traditionally been considered the gold standard for evaluating the HPA axis; however, its routine use in pediatric practice is limited by the risks and discomfort associated with induced hypoglycemia.[1] In addition, ITT is contraindicated in patients with cardiovascular disease or with a history of seizures.[2] Therefore, safer alternative dynamic tests are frequently used in children. The human corticotropin-releasing hormone test has been proposed as an acceptable method for assessing the HPA axis because it stimulates the axis at a higher anatomical level and induces endogenous adrenocorticotropic hormone (ACTH) secretion. In contrast, synthetic ACTH stimulation tests evaluate adrenal responsiveness to exogenous ACTH. Among these, the low-dose ACTH stimulation test (LDAT) is considered to provide a more physiological adrenal stimulus than the standard-dose test and has been suggested to be more sensitive for detecting CAI.[2]

Although several studies have proposed different threshold values for morning cortisol levels that may indicate a functionally intact HPA axis, there is no clear consensus.[3-5] In pediatric populations, LDAT is frequently required to assess adrenal function. The aim of this study was to retrospectively evaluate the demographic characteristics, indications for testing, and results of patients who underwent LDAT in a tertiary pediatric endocrinology center.

METHODS

This retrospective study included pediatric patients who underwent an LDAT at a tertiary pediatric endocrinology center between November 2023 and January 2026. The study was approved by the University of Health Sciences Türkiye, İzmir City Hospital Ethics Committee (approval no: 2026/225, date: 08.04.2026). Written informed consent was obtained from the parents or legal guardians of all participants. All procedures were conducted in accordance with the Declaration of Helsinki.

Demographic data, clinical characteristics, indications for testing, and laboratory findings were obtained from medical records. Weight, height, and body mass index standard deviation scores were calculated according to age- and sex-specific national reference data.[6]

Basal serum cortisol and ACTH measurements were obtained from routine blood samples collected during outpatient follow-up visits and analyzed in the hospital's central laboratory according to standard clinical practice. In contrast, baseline (0-minute) ACTH and cortisol samples were collected under standardized test conditions in a dedicated testing room by experienced healthcare personnel trained in endocrine stimulation testing, as part of the LDAT protocol. All blood samples during the LDAT procedure were collected at protocol-defined time points to ensure procedural consistency and the reliability of test results. Serum ACTH and cortisol concentrations were measured by electrochemiluminescence immunoassay on a Cobas® e801 analyzer (Roche Diagnostics, Mannheim, Germany).

The LDAT was performed according to standard protocols. All tests were conducted in the morning hours (typically between 08:00 and 09:00). A 1 µg dose of synthetic ACTH (Synacthen) was administered intravenously. To prepare the 1 µg dose, a 250 µg Synacthen ampoule was diluted to a final volume of 250 mL with normal saline, and 1 mL of this solution was used for testing. Serum cortisol levels were measured at baseline (0 minutes) and at 30 minutes after ACTH administration.[2] Although cortisol levels at 0 and 30 minutes were available for all patients undergoing LDAT, basal cortisol and ACTH measurements prior to testing were not obtained for all patients because of the retrospective nature of the study. A peak serum cortisol level ≥18 µg/dL at 30 minutes was accepted as an adequate adrenal response, whereas values <18 µg/dL were considered indicative of an insufficient cortisol response.[7] Patients were classified into adequate and insufficient response groups.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro-Wilk test. Continuous variables were tested for normality using the Shapiro-Wilk test and presented as mean ± standard deviation or median [interquartile range (IQR)], as appropriate.

Comparisons between groups (adequate vs. insufficient cortisol response) were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of cortisol levels measured at 0 minutes. The area under the curve (AUC) and the 95% confidence intervals (CIs) were calculated. The optimal cut-off value was determined based on the best balance between sensitivity and specificity. A p value <0.05 was considered statistically significant.

RESULTS

A total of 120 patients (61 females, 59 males) underwent the LDAT. The median age was 11.5 years (IQR: 5.6); 55.8% of the cohort were in the pubertal stage. The anthropometric characteristics and baseline ACTH and cortisol levels are summarized in Table 1.

The most common indications for testing were thyroid function abnormalities suggestive of central hypothyroidism (30.8%), followed by short stature or growth hormone deficiency (27.5%), intracranial mass lesions or malformations (21.7%), and other anterior pituitary hormone abnormalities (6.7%) (Figure 1). Less frequent indications included sepsis, prolonged steroid use, and hypoglycemia. Additional anterior pituitary hormone deficiencies were present in 30.3% of the patients. Among patients with additional anterior pituitary hormone deficiencies, growth hormone deficiency was the most frequently observed (57.9%).

An insufficient cortisol response was observed in 24.2% of the patients (n=29). No statistically significant differences were observed between the adequate and insufficient response groups with respect to age, sex, anthropometric characteristics, pubertal status, indications for testing,

or presence of additional anterior pituitary hormone deficiencies (all p>0.05) (Table 2).

A total of 37 patients underwent testing for suspected central hypothyroidism, 10 of whom exhibited an insufficient cortisol response. Among these patients, five were obese, two were malnourished, and three had a history of antipsychotic or antiepileptic drug use that was associated with thyroid function test abnormalities.

The mean serum cortisol level at 0 minutes was significantly higher in the adequate response group compared to the insufficient response group (9.02±3.73 µg/dL vs. 5.33±3.94 µg/dL, p<0.001).

ROC analysis demonstrated that cortisol levels at 0 minutes had a moderate ability to discriminate between adequate and insufficient cortisol responses (AUC =0.75, 95% CI: 0.65-0.85, p<0.001) (Figure 2). A cut-off value of 5.05 µg/dL yielded a sensitivity of 86.8% and a specificity of 55.2% for predicting an adequate cortisol response. Notably, none of the patients with a baseline cortisol level above 13 µg/dL exhibited an insufficient cortisol response during LDAT.

Table 1. Baseline characteristics of the study population

Variable	n	Median (IQR)
Age (years)	120	11.51 (5.59)
Weight (kg)	120	45.0 (47.15)
Weight SDS	120	0.25 (2.60)
Height (cm)	120	147.0 (36.15)
Height SDS	120	-0.25 (1.35)
BMI (kg/m²)	120	20.22 (9.51)
BMI SDS	120	0.57 (2.30)
Basal ACTH (pg/mL)	73	17.8 (13.97)
Basal cortisol (µg/dL)	89	6.9 (4.90)

IQR: Interquartile range, BMI: Body mass index, SDS: Standard deviation scores, ACTH: Adrenocorticotropic hormone

DISCUSSION

In this study, we evaluated the clinical characteristics, indications, and outcomes of LDAT in a pediatric population at a tertiary endocrinology center. In our cohort, the most frequent indication for testing was to rule out concomitant CAI in patients diagnosed with central hypothyroidism prior to the initiation of therapy. Although this is not a typical indication for LDAT, it may be explained by important clinical considerations. In patients with suspected central hypothyroidism, adrenal function should be evaluated before initiating levothyroxine replacement, as untreated concomitant adrenal insufficiency may precipitate life-threatening adrenal crisis following thyroid hormone therapy.[8] This issue is particularly relevant in pediatric endocrinology and may account for the high frequency with which LDAT was performed for this indication in our cohort. On the other hand, central hypothyroidism is a relatively rare endocrine disorder.[9] Furthermore, biochemical findings suggestive of central hypothyroidism—particularly low free T4 levels in the presence of normal or low TSH—may be influenced by multiple factors, including assay variability, laboratory interference, medication use, and timing of sampling, potentially leading to misinterpretation of thyroid function tests.[10-12] In our clinical practice, thyroid function tests are often repeated two or three times to confirm the persistence of abnormalities before making a definitive diagnosis. In patients with persistent findings suggestive of central hypothyroidism, LDAT was performed

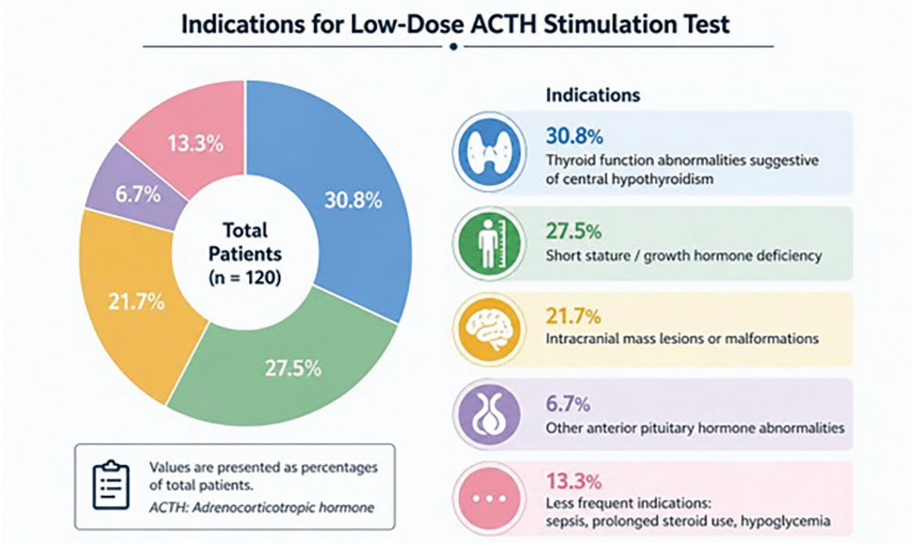

Figure 1. Distribution of indications for the low-dose ACTH stimulation test

to exclude concomitant CAI. In addition, free T4 levels are measured in our laboratory using immunoassay methods, which are known to be susceptible to analytical interference. In contrast, liquid chromatography-tandem mass spectrometry provides more accurate and specific measurements of thyroid hormones, particularly in the presence of assay-related limitations.[13] Taken together, these factors may contribute to the high frequency of suspected central hypothyroidism as an indication for LDAT in our cohort. The use of more accurate laboratory methods may reduce unnecessary testing in these patients, thereby minimizing both healthcare costs and the associated psychological burden on families.

Other frequent indications for testing included short stature/growth hormone deficiency, intracranial mass lesions or malformations, and other anterior pituitary hormone abnormalities, which further support the importance of evaluating adrenal function in these patients. Our findings demonstrate that LDAT is widely used across diverse clinical indications and that approximately one-quarter of patients exhibit an insufficient cortisol response. Consistent with previous reports, we found no significant differences between groups in terms of age, sex, or anthropometric parameters.[9,14] This suggests that clinical characteristics alone are insufficient to predict adrenal insufficiency, reinforcing the need for biochemical evaluation. Similarly, the presence of additional anterior pituitary hormone deficiencies did not differ significantly between groups, indicating that adrenal insufficiency cannot be reliably inferred, even in patients with multiple pituitary abnormalities.

One of the key findings of our study is that cortisol levels measured at 0 minutes were moderately predictive of adrenal sufficiency, with an AUC of 0.75. A cut-off value of 5.05 µg/dL provided high sensitivity (86.8%) but moderate specificity. This suggests that baseline cortisol measurements obtained at the time of testing may have clinical utility in identifying patients at low-risk of adrenal insufficiency, potentially reducing the need for dynamic testing in selected cases. However, the relatively low

Table 2. Comparison of clinical, anthropometric, and hormonal characteristics between patients with adequate and insufficient cortisol response

Variable	Adequate response (n=91)	Insufficient response (n=29)	p value
Age (years)	11.6 (5.34)	10.64 (5.45)	0.38[a]
Sex (female/male)	50/41	11/18	0.11[b]
Weight (kg)	45.24±28.39	45.58±34.1	0.46[b]
Weight SDS	0.5±1.87	0.31±1.65	0.90[b]
Height (cm)	147.8 (35.47)	134.0 (44)	0.66[a]
Height SDS	-0.30±1.18	-0.52±0.96	0.81[b]
BMI (kg/m²)	22.41±7.8	21.41±9.04	0.75[b]
BMI SDS	0.56±1.71	0.56±1.63	0.79[b]
Prepubertal/pubertal	33/58	9/20	0.93[b]
Etiology (%)			0.69[b]
-Suspected central hypothyroidism	29.7	34.5	
-Intracranial mass lesions or malformations	19.8	27.6	
-Short stature/growth hormone deficiency	29.7	20.7	
-Other anterior pituitary hormone abnormalities	5.5	3.4	
Additional pituitary hormone deficiency (%)	29.7	31.0	0.91[b]
Basal ACTH (n, 7.2-63.3 pg/mL) (n=73)	16.35 (13.36)	22.4 (8.4)	**0.03[b]**
Basal cortisol (µg/dL) (n=89)	6.57±3.78	6.01±3.37	0.34[a]
ACTH (0 min) (n, 7.2-63.3 pg/mL)	28.36±18.3	22.87±14.46	0.42[b]
Cortisol (0 min) (µg/dL)	9.02±3.73	5.33±3.94	**<0.001[b]**
Cortisol (30 min) (µg/dL)	20.1 (4.92)	13.9 (2.8)	**<0.001[a]**

Values are presented as mean ± standard deviation or median (interquartile range).

[a]Mann-Whitney U test

[b]Chi-square test or Student's t-test, as appropriate

BMI: Body mass index, SDS: Standard deviation scores, ACTH: Adrenocorticotropic hormone

specificity indicates that such an approach should be used cautiously and in conjunction with clinical judgment. These findings are in line with previous studies reporting considerable variability in the diagnostic performance of basal cortisol levels, with no universally accepted cut-off value.[2,4,5] Although higher morning cortisol levels (e.g., >13 µg/dL) are generally considered indicative of an intact HPA axis, values within the intermediate range typically require further evaluation with dynamic stimulation tests.[2,3] Notably, none of the patients in our cohort with a baseline cortisol level above 13 µg/dL exhibited an insufficient cortisol response during LDAT, further supporting the clinical utility of this threshold for excluding adrenal insufficiency.

Although basal ACTH levels were significantly higher in the insufficient response group, levels remained within the reference range in both groups. Similarly, basal cortisol levels obtained before testing did not differ significantly between groups, whereas concentrations measured at 0 minutes during LDAT were significantly higher in the adequate response group. These findings may be explained, at least in part, by differences in sampling conditions. Routine outpatient ACTH and cortisol measurements are susceptible to preanalytical factors, particularly variations in sampling time and collection procedures. In routine clinical practice, blood samples may be obtained at different times of the day and under varying conditions with respect to fasting status, recent physical activity, and psychological stress, all of which may influence cortisol concentrations. In contrast, ACTH and cortisol samples obtained during LDAT were collected under standardized conditions according to a predefined protocol. Notably, ACTH concentrations measured at the start of the stimulation test did not differ significantly between groups. In addition, the limited number of available basal ACTH and cortisol measurements may have reduced the statistical power of these comparisons. Therefore, the observed differences in routine basal hormone measurements should be interpreted cautiously and are unlikely to reflect clinically meaningful differences in HPA axis function. These findings further emphasize the importance of standardized sampling conditions when interpreting ACTH and cortisol levels.

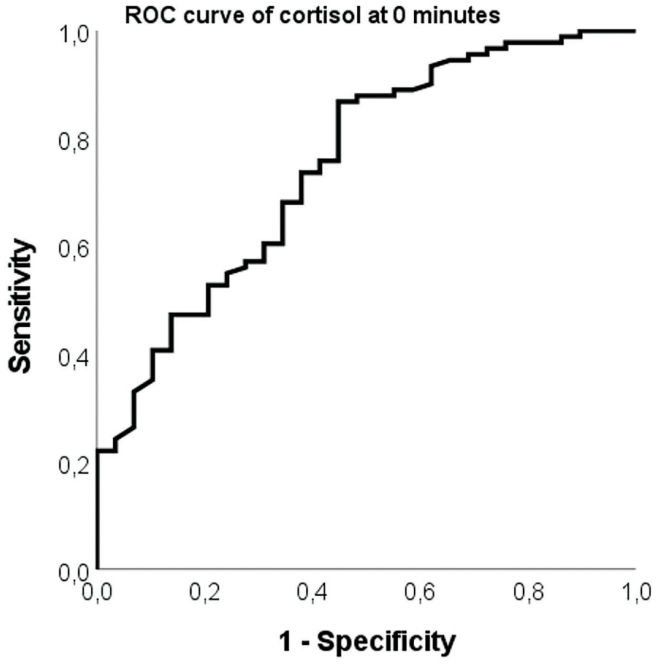

Figure 2. Receiver operating characteristic (ROC) curve of cortisol levels at 0 minutes for predicting adequate adrenal response

The use of a peak cortisol cut-off of 18 µg/dL is consistent with widely accepted clinical thresholds; however, assay-specific variability may influence optimal cut-off values. Recent studies have suggested that lower thresholds may be appropriate when using modern immunoassays or mass spectrometry-based methods.[2,15,16] Therefore, caution is warranted when generalizing cut-off values across different laboratory settings.

Study Limitations

This study has several limitations. First, its retrospective design may have introduced selection bias, reduced control over preanalytical conditions, and limited the availability of certain laboratory parameters, including basal ACTH and cortisol levels. In addition, the single-center nature of the study may limit the generalizability of the findings to other populations and clinical settings. Second, we did not perform confirmatory testing with ITT or other dynamic tests, which limits the ability to directly compare diagnostic performance. Third, assay-specific variability was not evaluated, and differences in cortisol measurement methods across laboratories may influence the applicability of the proposed cut-off values. Despite these limitations, our study provides valuable real-world data on the use of LDAT in a pediatric population and highlights the potential role of baseline cortisol levels obtained during testing.

CONCLUSION

LDAT remains a practical and widely used method for evaluating adrenal function in children. Baseline cortisol levels measured at the time of testing may provide useful clinical information for identifying patients at low-risk of adrenal insufficiency; however, dynamic testing remains essential for definitive diagnosis. Further prospective studies are needed to refine diagnostic thresholds and optimize testing strategies in pediatric populations.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, İzmir City Hospital Ethics Committee (approval no: 2026/225, date: 08.04.2026).

Informed Consent: Written informed consent was obtained from the parents or legal guardians of all participants.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: B.E.F., G.T.V., Concept: B.E.F., Design: B.E.F., B.N.D., Data Collection or Processing: G.T.V., Analysis or Interpretation: B.E.F., G.T.V., B.N.D., Literature Search: B.E.F., G.T.V., B.N.D., Writing: B.E.F., G.T.V., B.N.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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