Comparison of Cortisol Responses to Low Dose ACTH Stimuli and Injection Stress

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ABSTRACT:

Comparison of cortisol responses to low dose ACTH stimuli and injection stress

Objective: We performed a placebo-controlled, single-blind, pilot study to compare the efficacy and safety of low dose cosyntropin stimulation test with injection stress in the diagnosis of secondary adrenocortical insufficiency.

Material and Methods: Twenty-five patients (22 F, 3 M) with a low or normal plasma ACTH level, morning plasma cortisol level <15 µg/dL and an indication of cosyntropin stimulation test for secondary adrenocortical insufficiency were included in the study. On the first day, the test was performed with direct intravenous injection with 1 mL 0.9% saline. On the second day at the same time and with the same method, the test was repeated with 1 µg/mL cosyntropin solution. Immediately prior to the procedure, blood samples were taken from the cannula located in the other forearm for measurement of plasma cortisol at 30th and 60th minutes.

Results: The median ACTH value of the patients selected for the study was 22.10 pg/mL (14.00-29.30) and the mean plasma cortisol level was 6.71±2.00 µg/dL. The baseline plasma cortisol values measured at one minute in the placebo-controlled tests for comparison of injection stress with 1 µg cosyntropin stimulation were similar, but a significant statistically significant difference was found between plasma cortisol values measured at 30th and 60th minutes (p<0.001).

Conclusion: Injection stress, which occurs during intravenous injection, does not cause an increase in cortisol responses. However, 1 µg cosyntropin stimulation test at the physiological doses produces a significant cortisol response, especially at 30 minutes.

Keywords: Adrenal insufficiency, cortisol, injection stress stimuli, low-dose cosyntropin stimulation test

ÖZET:

Enjeksiyon stresi ve düşük doz ACTH uyarısında kortizol yanıtlarının karşılaştırılması

Amaç: Sekonder adrenal yetmezlik tanısında düşük doz kosintropin stimülasyon testinin etkinlik ve güvenilirliğini enjeksiyon stresi ile karşılaştırmak için plasebo kontrollü, tek kör, pilot bir çalışma yaptık.

Gereç ve Yöntemler: Çalışmaya plazma ACTH değeri düşük ya da normal, sabah plazma kortizol değeri <15 µg/dL olan ve sekonder adrenal yetmezlik tanısı için kosintropin uyarı testi endikasyonu olan 25 hasta (22 K, 3 E) alındı. İlk gün test, 1 mL %0.9 serum fizyolojik ile direkt intravenöz enjeksiyon şeklinde yapıldı. İkinci gün aynı saatte ve yöntemle test 1 µg/mL kosintropin solüsyonu ile tekrarlandı. İşlemden hemen önce, 30 ve 60. dakikalarda plazma kortizol ölçümü için diğer ön kolada hazır bulunan kanülden kan örnekleri alındı.

Bulgular: Çalışmaya seçilen hastaların medyan ACTH değeri 22.10 pg/mL (14.00-29.30), ortalama plazma kortizol değeri 6.71±2.00 µg/dL idi. 1 µg kosintropin stimulasyonu ve enjeksiyon stresinin karşılaştırılması için yapılan plasebo testte 0. dakikada ölçülen bazal plazma kortizol değerleri benzer iken, 30. ve 60. dakikada ölçülen plazma kortizol değerleri arasında belirgin şekilde istatistiksel anlamlı fark saptandı (p<0.001).

Sonuç: İntravenöz enjeksiyon esnasında oluşan enjeksiyon stresi kortizol yanıtlarında artışa sebep olmamaktadır. Oysa fizyolojik dozdaki 1 µg kosintropin stimulasyonu özellikle 30. dakikada belirgin bir kortizol yanıtı oluşturur.

Anahtar kelimeler: Adrenal yetmezlik, kortizol, enjeksiyon stres uyarısı, düşük doz kosintropin stimülasyon testi

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INTRODUCTION

Adrenal insufficiency (AI), may occur from the impairment of primary function of the adrenal glands, insufficiency of adrenocorticotropic hormone (ACTH) secretion from hypothalamic-pituitary diseases or sudden withdrawal of chronic steroid therapy. AI diagnosis is usually simple in the presence of clinical and laboratory findings. However, recognition of subclinical cases is more problematic and early recognition of these forms is crucial. AI is a life-threatening condition when adequate replacement therapy is not performed. Subclinical cases usually have normal basal cortisol and ACTH levels, and many symptoms (anorexia, weight loss, fatigue, etc.) are insidious and uncertain (1).

Cortisol secretion follows a circadian pattern; the highest concentrations are observed in the morning (at around 6:00) and range from 10 to 20 µg/dL. Low morning serum cortisol concentration (<3µg/dL) strongly supports AI and AI is excluded in almost all patients when morning serum cortisol concentration is >15 µg/dL. However, serum cortisol levels between 3 and 15 µg/dL do not show neither adrenal insufficiency nor adequacy (2).

The diagnosis of AI is based on the demonstration of inappropriately low cortisol production, and prompt diagnosis is important to remove the risk of acute adrenal crisis in patients with suspected AI. A number of dynamic tests can be performed to evaluate the hypothalamic-pituitary-adrenal (HPA) axis. But there is still a considerable debate regarding which is the best.

The insulin tolerance test (ITT) was accepted as the "gold standard" for detecting secondary adrenal insufficiency. However, the use of this test requires careful supervision and experience. In addition, because of its side effects and contraindications, other screening tests that are simpler, safer and more reliable are needed (3,4). The traditional [250 µg tetracosactide, ACTH-(1-24)] cosyntropin stimulation test (CST) which is performed with administration of 250 µg ACTH intravenously is frequently criticized for being at supraphysiological doses and with its suboptimal sensitivity and may dismiss mild adrenal insufficiency cases (5,6). In recent years, it has been

shown that the 1 µg ACTH (Low-Dose Cosyntropin Stimulation Test, LDCST) test has a high specificity and sensitivity for the assessment of central AI in patients with hypothalamic-pituitary disorders and its strong correlation with ITT (6-8). However, in many studies it is still suggested that the LDCST is more sensitive than the 250 µg ACTH test, but this condition is controversial (6).

LDCST has a more physiological dose compared to CST. However, there is no study comparing this dose with physiological stress. For this reason, in this study, we aimed to compare only the serum cortisol responses to injection stress and to LDCST.

MATERIAL AND METHOD

Patient Selection

Twenty-five patients (22 F, 3 M) between the ages of 21 years and 60 years who were admitted to the Endocrinology and Metabolic Diseases Outpatient Clinic with an indication of cosyntropin stimulation test found at the clinical/laboratory evaluation were included in the study. Preexisting hypophyseal disease [operated hypophysis adenoma (n=4), prolactinoma (n=3), acromegaly (n=2), empty sella (n=3), partial hormone deficiency (n=1), microadenoma (n=1)] were seen in 14 of the patients, and 12 had cortisol levels of <10 µg/dL and accompanying symptoms. Pregnancy, breastfeeding, serious comorbidities and patients still receiving steroid therapy were not included in the study.

Design

Our study was planned as a cross-sectional, placebo-controlled, single-blind pilot study.

Tests were conducted in two consecutive days AT around 8:00-9:00 am, following 8-12 hours of fasting. On the first day, a cannula was placed in the forearm of the patient to assess injection stress and from the other forearm, 1 mL 0.9% saline was injected via direct intravenous injection. This was follwed by 2 mL of 0.9% saline injection. On the second day, a cannula was placed in the forearm of the patient in a similar manner for LDCST and 1 mL of 1 µg/mL

cosyntropin solution was given via direct intravenous injection from the other forearm. This was followed by injection of 2 mL %0.9 saline. Blood samples were taken from the other forearm for measurement of plasma cortisol at 30th and 60th minutes, immediately before the application of saline and cosyntropin. During the test, the patients were kept in sitting position.

Preparation of 1 µg cosyntropin solution: 1 µg/mL cosyntropin solution was obtained by adding 1 ampoule of Synacthen 250 µg/1 mL (Sigma-Tau Industrie Farmaceutiche Riunite S.p.A. Viale Shakespeare, 47 00144 Rom, Italien) into 249 mL 0.9% sterile saline. The present solution was kept in the refrigerator for 2 months at 4 ℃ - 8 ℃.

Cortisol levels were measured by immunoassay method using the Access Cortisol kit (Beckman Coulter, Inc. USA) with Beckman Coulter UniCel DxI 800 automatic analyzer, ACTH levels were measured by the immunometric chemiluminescence method with the Immulite 2000 XPI (Siemens) automatic analyzer using the ACTH kit (ACT Immulite 2000 systems, Siemens, UK). Adequate peak cortisol levels at 30th and 60th minutes after LDCST were taken as ≥18 µg/dL.

Approval was obtained from the local ethics committee before the study. Information was provided to all the participants about the procedure and written informed consent was obtained from those who agreed to participate.

Statistical Analysis

Statistical analyzes were performed using the IBM SPSS (Statistical Package for the Social Science, version 17.0) program. Data were given as mean ± standard deviation (SD) for normal distribution, and as median value and values between 25% and 75% quartiles for non-normal distribution. The normal distribution of the data was assessed by the Kolmogorov-Smirnov test. The paired-t test was used to compare data. A p value of <0.05 was accepted as significant for all statistical analyzes.

RESULTS

Twenty-five (22 F, 3 M) patients with suspected secondary adrenal insufficiency were included in the study. The mean age of the patients was 44.74±9.70 years. The pre-test median ACTH and mean plasma cortisol levels were 22.10 pg/mL (14.00-29.30) and 6.71±2.00 µg/dL, respectively (Table-1). The baseline plasma cortisol values measured at 0th minute in the placebo test for comparison of 1 µg cosyntropin stimulation and injection stress were similar, while there was a statistically significant difference was

Table-1: Demographic and biochemical characteristics of patients

Age (years)	44.74±9.70
Gender (F/M)	22/3
Basal Cortisol, µg/dL	6.71±2.00
ACTH*, pg/mL (IQR**)	22.10 (14.00-29.30)

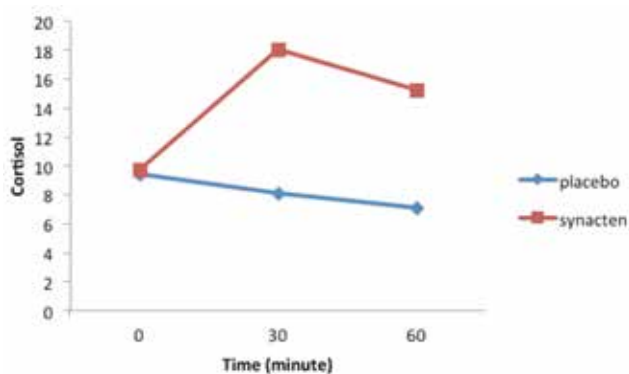*ACTH: Adrenocorticotropic Hormone, **IQR= Between 25% and 75% quartiles

Figure-1: Cortisol responses according to time in placebo and 1 µg of cosyntropin test

Table-2: Comparison of 1 µg cosyntropin stimulation test results with placebo

	LDCST‡	Placebo	p value
0.min Cortisol (µg/dL)	9.69±3.95	9.29±3.56	0.459
30.min Cortisol (µg/dL)	18.38±3.80	6.88±2.19	<0.001
60.min Cortisol (µg/dL)	15.27±4.06	7.89±2.37	<0.001

‡LDCST: Low-Dose Cosyntropin Stimulation Test

found between the plasma cortisol values measured at 30th and 60th minutes (p<0.001) (Table-2 and Figure-1).

Adequate cortisol response was obtained in 68% (n=17) of patients at 30th minute and in 28% (n=7) at 60th minutes following LDCST when we took the cut off value for peak cortisol level as ≥18 µg/dL. In eight patients, adequate cortisol response could not be obtained after LDCST. No cortisol response exceeding this threshold was found in any of the patients at the 30th and 60th minutes after the placebo test in which the injection stress was assessed.

DISCUSSION

Adrenal insufficiency, of which the early diagnosis is crucial, is a disease that may persist with nonspecific symptoms, as it may also cause serious clinical symptoms (hypotension, shock, electrolyte disturbances, etc.) in stress situations. Cosyntropin stimulation tests are commonly used to diagnose AI. However, the appropriate cosyntropin dose to assess hypothalamic–pituitary–adrenal (HPA) axis in the case of suspected adrenal insufficiency is still controversial.

CST measures the direct adrenal reserve and is the most commonly used test to identify primary or extended secondary adrenal insufficiency (9). However, because the pharmacological dose of ACTH is used in CST, it is supraphysiological (3). This test leads to ongoing debate on whether it predicts early secondary adrenal insufficiency accurately or not. Although patients sometimes respond normally to CST, they may produce an abnormal response to ITT, which is accepted as the gold standard test for evaluation of the HPA axis (5). Therefore, LDCST is especially recommended for the diagnosis of secondary adrenal insufficiency (6,10). In addition, it also has additional advantages such as the absence of side effects of LDCST, its cost-effectiveness and being easily available for the exclusion of adrenal insufficiency in outpatient clinics (11).

Although there is no consensus on which diagnostic test may be optimal, several meta-analyzes have acknowledged and encouraged LDCST to show higher sensitivity, especially in moderate to mild adrenal insufficiency (12-14). However, the absence of commercially available 1 µg cosyntropin preparations results in different ACTH formulations to be used in different studies and concerns that arise from different dilution techniques.

The cortisol in humans is released at a circadian rhythm, with a maximum level at 7:00-9:00 in the morning and a lowest level at midnight. However, cortisol is a stress hormone which is stimulated by serious cardiovascular, infectious, and by a degree of psychological stress (4). Intravenous injection also causes a physiological stress. In fact, this stress may be intense that at some point, it may cause syncope. 1 µg of cosyntropin is the physiological stress dose. Some studies investigating the effect of LDCST administration technique have shown that the drug preparation technique, storage conditions, the length of the plastic tube used, and test administration time may have an impact on cortisol responses (15-17). However, in our literature review we did not find a study that evaluated the cortisol response in injection stress and compared LDCST with such physiological stress.

The physiological injection stress that occurred during the intravenous injection in our study did not lead to a significant increase in cortisol responses during the test. On the other hand, LDCST performed with 1 µg/mL cosyntropin solution at appropriate preparation and storage conditions showed a significant cortisol response at 30th and 60th minutes compared to the placebo test. In addition, we found that the 30th minute peak cortisol response was higher in our study, which was consistent with the literature (14,18,19). Saiegh et al. (16) showed that 60th minute serum free cortisol alue was higher than the 30th minute value in 10-35% of cases in healthy volunteers. In our study, however, no cortisol response was obtained at 60th minute in any patient in whom we could not receive a peak cortisol response at 30th minute.

This placebo-controlled study was conducted in patients with suspected adrenal insufficiency. It might

have been more appropriate to perform this study among healthy volunteers. However, the results obtained show that the 1 μg cosyntropin stimulation test is highly effective in assessing the diagnosis of secondary adrenal insufficiency.

As a result; LDCST performed with 1 μg/mL cosyntropin solution is highly effective in assessing the diagnosis of sec-ondary adrenal insufficiency and physiological injection stress occuring during the test does not cause significant cortisol increase.

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