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Araştırma Makalesi / Research Article

Determination of Cortisol Peak Response Time in ACTH Stimulation Test

ACTH Uyarı Testinde Kortizol Pik Yanıtı Zamanının Belirlenmesi

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Abstract

Adrenocorticotropic hormone (ACTH) stimulation test is widely used in diagnosis of adrenal insufficiency. Synthetic ACTH analogue has two formulations: Short-acting intravenous or intramuscular form and intramuscular depot form. In the standard ACTH stimulation test protocol, 250µg synthetic ACTH (1-24) is administered intravenously. Blood samples are then taken at 0, 30, 60 and 90 minutes for cortisol. We aimed to determine the time of cortisol peak response by evaluating cortisol responses to intramuscular depot ACTH. Seventy patients who underwent ACTH stimulation test between October 2017 and February 2019 in Eskişehir Osmangazi University Endocrinology Department were included to the study. 250 mcg intramuscular depot ACTH was administered between 08.00-09.00 a.m. Blood samples were taken before ACTH administration and at 30, 60, 120, 240 minutes. Cortisol levels 18 mcg/dl and above was accepted as normal response. Since 20 mcg / dl and above are accepted as normal response in different publications, evaluation was also made on this value. The highest cortisol level was reached in 240 minutes in all patients. If normal cortisol response is accepted as 20 mcg/dl and above, 38 patients reached normal cortisol values at 30th minute, 12 patients at 60th minute, 6 patients at 120th minute. If normal cortisol response is accepted as 18 mcg/dl and above, 38 patients reached normal cortisol values at 30th minute, 10 patients reached normal cortisol values at 60th minute, 2 patients reached 120th minute. In both groups, only one patient was able to reach the target cortisol value after prolongation to 240 minutes. We suggest that prolongation of the stimulation test with intramuscular depot ACTH up to 120 minutes may prevent patients from being misdiagnosed with adrenal insufficiency, but prolongation to 240 minutes will not provide additional benefit.

Keywords: Adrenal insufficiency, ACTH, cortisol, cosyntropin

Özet

Adrenal yetmezlik tanısında Adrenokortikotropik hormon (ACTH) stimülasyon testi yaygın olarak kullanılmaktadır. Sentetik ACTH analoğunun iki formülasyonu bulunmaktadır: intravenöz veya intramüsküler kullanımı için kısa etkili form ve intramüsküler kullanım için depo formu. Standart ACTH stimülasyon test protokolünde, 250µg sentetik ACTH (1-24) intravenöz uygulanır. Sonrasında 0, 30, 60 ve 90. dakikalarda kortizol için kan örnekleri alınır. Bu çalışmamızda intramüsküler uygulanan depo ACTH testine kortizol yanıtlarını değerlendirerek kortizol pik yanıtının zamanını belirlemeyi amaçladık. Çalışmaya Eskişehir Osmangazi Üniversitesi Endokrinoloji ve Metabolizma Hastalıkları Bilim Dalında Ekim 2017- Şubat 2019 tarihlerinde ACTH stimülasyon testi uygulanmış 70 hasta alındı. Hastalara 250 mcg depo ACTH 08.00-09.00 saatleri arasında intramuskuler olarak uygulandı. Kan örnekleri ACTH uygulaması öncesi ve 30, 60, 120 ve 240. dakikalarda alındı. Kortizol değeri 18 mcg/dl ve üzeri normal yanıt olarak kabul edildi. Farklı yayınlarda 20 mcg/dl ve üzeri de normal yanıt olarak kabul edildiğinden bu değer üzerinden de değerlendirme yapıldı. Hastaların hepsinde en yüksek kortizol seviyesine 240. dakikada ulaşıldı. Normal kortizol yanıtı 20 mcg/dl ve üzeri kabul edildiğinde 38 hasta 30. dakikada, 12 hasta 60. dakikada, 6 hasta 120. dakikada, normal kortizol değerine ulaştı. Normal kortizol yanıtı 18 ve üzeri kabul edildiğinde 38 hasta 30. dakikada, 10 hasta 60. dakikada, 2 hasta 120. dakikada normal kortizol değerine ulaştı. Her iki grupta da sadece birer hasta 240. dakikaya uzatıldığında hedef kortizol değerine ulaşabildi. Sonuç olarak intamüsküler uygulanan depo ACTH ile stimulasyon testinin 120. dakikaya kadar uzatılmasının hastaların yanlışlıkla adrenal yetmezlik tanısı almasını engelleyebileceği, 240. dakikaya kadar uzatılmasının ise ek fayda sağlamayacağı görüşündeyiz.

Anahtar Kelimeler: Adrenal yetmezlik, ACTH, kortizol, kosintropin

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1. Introduction

Despite attempts for improvement, the diagnosis of adrenal insufficiency remains a problem and the diagnosis are often delayed (1). Although various diagnostic methods are available (e.g. insulin tolerance test, metyrapone test, high-dose Adrenocorticotropic hormone (ACTH) stimulation test, low-dose ACTH stimulation test, morning cortisol) there is still debate about which test should be preferred (2).

ACTH stimulation test is the most widely used test for the evaluation of adrenal insufficiency. The synthetic ACTH analog has the amino acid sequence 1-24 of the human ACTH 1-39 molecule and constitutes the overall biological activity of the ACTH 1-39 molecule; often known as cosyntropin or tetracosactide (3). Tetracosactide is available in two formulations: a short-acting form for intravenous or intramuscular use and a depot form for only intramuscular use (4). The depot formulation contains inorganic zinc complexes that adsorb the active ingredient and result in a long release. The intravenous form of synthetic ACTH is not available in countries where depot tetracosactide is the only synthetic ACTH form.

In general, the standard ACTH stimulation test protocol involves taking blood samples for cortisol at 0, 30, 60 and 90 minutes after intravenous administration of 250 µg synthetic ACTH 1-24 (3). Previous studies suggest that a single value after 30 minutes may adequately reflect the adrenocortical response (5, 6). Other reports suggested evaluation after 60 minutes to provide an accurate estimate of adrenal function (7). Recent studies have shown that termination of the test at the 60th minute may give false concordance with adrenal insufficiency in 11% of cases (3). Because of the presence of depot tetracosactide in our country, standard ACTH test is applied intramuscularly in our clinic. Depot Synacthen® test, which is widely used in the diagnosis of secondary adrenal insufficiency, takes longer time (8).

In this study, we aimed to determine the time of cortisol peak response by evaluating cortisol responses to the depot ACTH applied intramuscularly.

2. Material and Method

This is a cross-sectional study in which data were collected retrospectively from patient files. All patients (70 patients) who underwent ACTH stimulation test in Eskisehir Osmangazi University Endocrinology and Metabolic Diseases Clinic between October 2017 and February 2019 were included in the study. Sixty patients were female and 10 were male. We collected the data of the patients who were planned to undergo ACTH stimulation test due to suspected adrenal insufficiency or suspected late onset congenital adrenal hyperplasia.

According to the protocol, 250 mcg depot ACTH (tetracosactide β 1-24, Synacthen®) was administered intramuscularly between 08.00-09.00 a.m. following fasting overnight. Blood samples were taken immediately before ACTH administration and at 30, 60, 120, 240 minutes. Serum cortisol levels were measured by electro-chemiluminescence immunoassay (ECLIA) technique. Baseline cortisol level was accepted as normal response above 18 mcg/dl when evaluating the results of the cases. In literature, some researchers accepted basal cortisol values as 20 mcg/dl and above. Therefore, we performed two separate analyzes for both cut-off values.

Data were compared statistically with IBM SPSS Statistics 21.0 program.

3. Results and Analysis

Ten of the patients included in the study were male and 60 were female. The average age of the group was 32.8 years old (29.7 for women and 51.5 for men). Twenty-seven patients had suspicion for adrenal insufficiency and 43 patients had suspicion for late-onset congenital adrenal hyperplasia.

The highest cortisol level was reached at 240 minutes in all patients. When normal cortisol response was accepted as 20 mcg/dl, 12 patients (17.1%) showed normal cortisol response at 0 minutes. 38 patients (54.3%) at 30 minutes; 12 patients (17.1%) at 60 minutes; six patients (8.6%) reached normal cortisol levels at 120 minutes. Only one patient (1.4%) reached normal cortisol levels at 240 minutes. In one patient, the target cortisol level could not be reached. When normal cortisol response is accepted as 20 mcg/dl and above, the results are shown in Table 1.

Table1. Peak Cortisol Response Times When Normal Cortisol Response ≥20 mcg / dl and Above Accepted

Cortisol value (≥20 mcg/dl)	Group with suspectedCAH (n, %)	Group with suspected AI (n, %)	Total (n, %)
0. min	11 (25,6 %)	1 (3,7 %)	12 (17.1%)
30. min	26 (60,5 %)	12 (44,4 %)	38 (54.2 %)
60. min	5 (11,6 %)	7 (25,9 %)	12 (17.1 %)
120. min	1 (2,3 %)	5 (18,5 %)	6 (8.6 %)
240. min	0	1 (3,7 %)	1 (1.5 %)
Not reached target	0	1 (3,7 %)	1 (1.5 %)
Total	43	27	70

CAH: Congenital Adrenal Hyperplasia, AI: Adrenal Insufficiency

When normal cortisol response was accepted as 18 mcg/dl and above, 19 patients (27.1%) had a cortisol level of 18 mcg/dl and above at zero point. 38 patients (54.2%) reached normal cortisol levels at 30 minutes; 10 patients (14.2%) at 60 minutes; two patients (2.85%) at 120 minutes. No patient reached normal cortisol at 240 minutes. One patient did not reach the target cortisol value. Table 2 shows the results when normal cortisol response is accepted as 18 mcg/dl and above.

Table 2. Peak Cortisol Response Times When Normal Cortisol Response ≥18 mcg / dl and Above Accepted

Cortisol value (≥18 mcg/dl)	Group with suspected CAH (n, %)	Group with suspected AI (n, %)	Total (n, %)
0. min	18 (41,9 %)	1 (3,7 %)	19 (27.1%)
30. min	22 (51,2 %)	16 (59,3 %)	38 (54.2%)
60. min	3 (7,0 %)	7 (25,9 %)	10 (14.2%)
120. min	0	2 (7,4 %)	2 (3%)
240. min	0	0	0
Not reached target	0	1(3,7 %)	1 (1.5 %)
Total	43	27	70 (100%)

CAH: Congenital Adrenal Hyperplasia, AI: Adrenal Insufficiency

4. Discussion and Conclusion

Because of the pulsatile and circadian release of cortisol, measuring basal cortisol levels is not a reliable method to evaluate the hypothalamic-pituitary-adrenal (HPA) axis (9). ACTH stimulation test has been used for many years to evaluate the HPA axis (8). Applied synthetic ACTH (1-24) shows all of the biological effect found in natural ACTH (1-39).

In standard dose ACTH test; blood is drawn for a serum cortisol sample before the intravenous or intramuscular injection of 250 mcg ACTH and after the injection at the 30th and 60th minutes. ACTH at this dose is considered to reach the plasma ACTH concentration at the pharmacological dose after 60 minutes.

However, questions about whether the termination of the test at the 60th minute may cause some patients to be mistakenly diagnosed with adrenal insufficiency, have not been clarified. In our study, we aimed to answer the question whether prolongation of the standard dose ACTH stimulation test up to 4 hours would provide additional benefit. In the results of our study, the highest cortisol level was reached at the 4th hour in all patients.

However, when the target cortisol level was taken as 18 mcg/dl and above, normal cortisol response was obtained in 67 (95.7%) of the 70 patients at 60 minutes. Two patients reached the target cortisol level at 120 minutes. No patient reached the target cortisol level at 240 minutes. And, normal cortisol response could not be achieved in one patient, in this group. When the target cortisol value was taken as 20 mcg/dl, normal cortisol response was obtained at 60 minutes in 62 (88.5%) of 70 patients. 6 patients achieved normal cortisol response at 120 minutes and 1 patient at 240 minutes. Normal cortisol response could not be achieved in one patient.

There are not many studies in the literature investigating the level of peak cortisol after ACTH application (10). In studies, it usually compares low and high dose applications of ACTH. In the study conducted by Younas et al., the 30th and 60th minutes were compared and it was concluded that the 30th minute cortisol levels were not significant (11). In order for the ACTH stimulation test to be significant, at least 60 min. cortisol level should be measured (12).

In the study of Kelestimur et al., the peak cortisol level was reached in 90th minute in the first group tested with 250 mcg ACTH, whereas the peak cortisol response in the second group tested with 1000 mcg ACTH was taken in 120th minute (8). In a study conducted by Longui et al., after application of intramuscular ACTH, 30th and 60th minute blood samples were taken and peak cortisol value was reached at 60th minute (9). In our study, when the target cortisol value was accepted as 20 mcg/dl and above, it was shown that termination of the test at the 60th minute may cause 7 patients (10%) to be diagnosed with adrenal insufficiency by mistake. If the target cortisol level is accepted as 18 mcg/dl, the termination of the test at the 60th minute will result in 3 patients (4.2%) being mistakenly diagnosed with adrenal insufficiency. Since the target cortisol level was reached at 240 minutes in only one patient, we believe that extending the test to the 4th hour does not provide additional benefit. As a result, stimulation test with depot ACTH which is applied intramuscularly is a frequently used test in the diagnosis of adrenal insufficiency. Prolonging the test up to 120 minutes may prevent patients from mistakenly diagnosed with adrenal insufficiency, however extending the test to 240 minutes would not provide additional benefit.

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