Adrenocorticotropin Stimulation Test in Congenital Adrenal Hyperplasia : Comparison between Standard and Low Dose Test†

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Abstract

The aim of the study was to compare the response between the standard and low dose adrenocorticotropin (ACTH) test for patients with congenital adrenal hyperplasia (CAH). The authors employed a 2-by-2 crossover design and enrolled 16 patients, 14 girls and 2 boys, aged between 1.4 months and 15 years. Steroid treatment was stopped 24 hours before each test was conducted. The standard ACTH (250 μg) test was performed followed by the low dose test (1 μg) in eight patients; the other eight underwent the low dose ACTH test first followed by the standard one. The cortisol and 17-hydroxyprogesterone (17-OHP) levels in each patient varied unpredictably between the two tests. The cortisol responses to the low dose ACTH at 30 and 60 minutes were lower than at time zero; in contrast to the 60-minute peak cortisol response to the standard dose. The serum 17-OHP in all specimens was more than 10,000 ng/dl (300 nmol/L), with the peak response at 60 minutes in both groups. Both the low dose and standard dose ACTH test indicated adrenal insufficiency and the high 17- OHP levels were diagnostic of 21- hydroxylase (21-OH) deficiency. If the low dose ACTH test becomes the "standard" ACTH test, the diagnosis of 21-OH deficiency would probably not be missed.

Key word : Congenital Adrenal Hyperplasia, Low Dose ACTH Test, 21-Hydroxylase Deficiency

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis. It is one of the most common causes of adrenal insufficiency in the pediatric age group. Disorders of 21-hydroxylation account for over 95 per cent of CAH cases. The severe form, due to diminished aldosterone and cortisol synthesis, manifests as a salt-wasting crisis in the first 2 weeks of life. Affected females have genital ambiguity due to the conversion of steroidal precursors upstream from 21-hydroxylase to androgens; affected males have normal male genitalia. The principle substrate, 17-hydroxyprogesterone (17-OHP), will have an elevated base level and will hyper-respond to ACTH in well-characterized patterns[1]. The standard (250 μg) short ACTH test is well established in the diagnosis of adrenal insufficiency. Studies in healthy subjects revealed that the standard 250 μg dose exceeds the adrenal cortex dose-response curve, and that a dose as low as 0.5 to 1.0 μg can elicit the same maximum response in 20 to 30 min[2,3] while in the standard test the peak values occurred at 60 or 120 min[4,5]. Indeed, the low dose test proved even more efficient in detecting secondary adrenal insufficiency than the standard test[2,6-11]. For primary adrenal insufficiency, low dose test also has a high diagnostic sensitivity and specificity[12-14].

The aim of the present study was to compare the cortisol and 17-OHP response to the 250 μg *vs* the 1 μg ACTH tests in patients with CAH to determine whether the 1 μg test can replace the 250 μg one in this group of patients.

SUBJECTS AND METHOD

Subjects

The study group consisted of 16 CAH children (14 girls and 2 boys) with a mean age of 4.6 yr (range 0.1-15.0). The children all manifested salt-wasting symptoms with hyponatremia, hyperkalemia and metabolic acidosis during the first 2 months of life. The 14 genetic females had pigmented ambiguous genitalia without palpable gonads while the two genetic males, siblings of 2 of the females, had normal male genitalia. During the study period, cortisone acetate was not available, so all the subjects received replacement therapy with prednisolone equivalent to glucocorticoid 20-30 μg/m^2/day and fludrocortisone acetate (Florinef®) 0.05-0.15 mg daily.

Study protocol

The 16 CAH children were randomly assigned to two sequences of the test 24 hours after withdrawal of steroid treatment. The first half received the standard followed by the low dose ACTH test while the second half received the low followed by the standard dose ACTH test. At least 7 days were allowed between tests. Serum cortisol and 17-OHP were assessed at 0, 30 and 60 min after ACTH infusion. All children aged below 5 yr were admitted 24 hr before each test for close monitoring of salt-wasting.

The Research Ethics Committee of Khon Kaen University Hospital approved the study, and the parents of the children gave informed written consent.

Standard and low dose ACTH test, cortisol and 17-OHP determination

The standard ACTH test was 250 μg ACTH (Cortrosyn, N.V Organon Oss Holland) intravenously, whereas low dose was 1 μg. The low dose was freshly prepared by diluting 250 μg ACTH with normal saline. The basal blood samples for serum cortisol (Cortisol RIA kit, ICN Biomedicals, Inc. CA, USA) and 17-OHP (Active ™17α-OH Progesterone RIA DSL-5000, Diagnostic Systems Laboratories, TX, USA) were taken at time 0. After the ACTH injection, the serum samples for cortisol and 17-OHP determinations were drawn at 30 and 60 min. The ACTH tests were performed between 08:00-10:00.

Statistical analysis

The authors performed a standard analysis for a 2-by-2 cross-over design. Due to evidence of a significant carry over effect, only the data from the first period was analyzed. By comparing the two study groups at each measurement time, the mean differences and their 95 per cent confidence intervals and p-value using *t*-test were calculated. The median changes between each combination of two time points "within patients" and compared "between groups" were also calculated by estimating the median differences and their 95 per cent confidence intervals (CI) and p-value using Mann-Whitney-U-test. To test for the difference in overall changes between the two groups, a random effect generalized linear model using generalized estimating equations (GEE) was used. A p-value of < 0.05 was required for significance. Stata (StataCorp, College Station, TX, USA) was the software used for all analyses.

RESULTS

Eight CAH patients (7 girls, 1 boy) aged 0.1-12.0 yr (median 2.5 yrs) received the low dose ACTH, followed by the standard dose test. The second group, comprising 8 patients (7 girls, 1 boy) age 0.1-15.0 yr (median 4.5 yrs), received the standard dose followed by the low dose test. The cortisol and 17-OHP levels in each patient varied unpredictably between the two tests. It was decided to analyze the data from only the first period due to evidence of a significant carry over effect. Fig. 1 shows the baseline serum cortisol at 0 min, 30 and 60 min in response to the low and standard dose tests. Cortisol responses (mean ± SD) to the low dose test at 0, 30, and 60 min were 11.34 ± 2.17, 10.99 ± 2.01 and 8.86 ± 1.82 μg/dl respectively. For the standard test, the responses were 11.94 ± 2.14, 13.58 ± 2.57 and 14.31 ± 2.32 μg/dl respectively (Table 1). The difference between the two groups at 60 min was two fold greater than at 30 min though not statistically significant. Table 2 presents the median changes of serum cortisol from one sample time to another. The greatest change from one time to another between the two groups was between 0 and 60 min. Fig. 2 shows the serum 17-OHP baseline values at 0 min, 30 and 60 min after the low and standard dose tests, all specimens were >10,000 ng/dl (300 nmol/L). The mean ± SD of serum 17-OHP responses to the low dose ACTH test at 0, 30, and 60 min were 21,797.2 ± 4,541.7, 24,519.8 ± 5,706.6, and 25,513.6 ± 6,035.4 ng/dl respectively. For the standard test, the 17-OHP responses were 34,344.5 ± 7,044.9, 33,821.1 ± 6,640.4 and 36,607.6 ± 7,157.6 ng/dl respectively (Table 3).

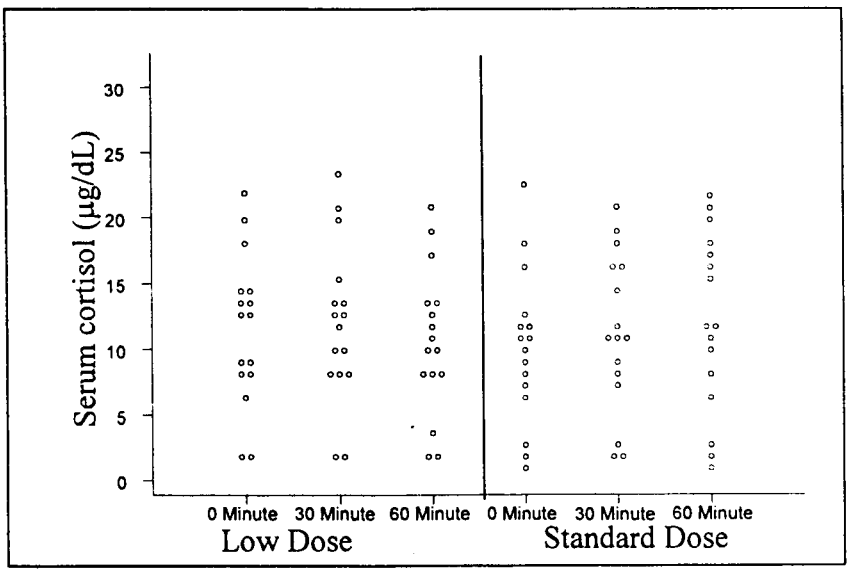

Fig. 1. Comparing serum cortisol (μg/dl) at 0, 30, and 60 minutes between CAH patients on the low (1 μg) *vs* the standard dose (250 μg) ACTH test (8 patients for each group).

Table 1. Serum cortisol (μg/dl) response to low (1 μg) and standard dose (250 μg) ACTH stimulation test at 0,30 and 60 min in 16 CAH patients (n = 8, each group), and the difference between the two groups.

Time	Low dose ACTH test (Mean ± SD)	Standard ACTH test (Mean ± SD)	Difference (Standard-low)	Confidence interval 95%	P-value*
0 min	11.34 ± 2.17	11.94 ± 2.14	0.60	-5.94 to 7.10	0.847
30 min	10.99 ± 2.01	13.58 ± 2.57	2.59	-4.42 to 9.59	0.441
60 min	8.86 ± 1.82	14.31 ± 2.32	5.45	-0.88 to 11.78	0.086

* *t*-test

Table 2. Changes (in median) of serum cortisol from 0 to 30 and 60 min and from 30 to 60 min, and the difference of the changes between the low and standard dose ACTH test in CAH patients.

Time	Low dose ACTH	Standard dose ACTH	Differences of the Changes (Standard-low ACTH)	P-value*
0 to 30 min	-0.2	0.50	0.70	0.046
0 to 60 min	-0.9	2.45	3.35	0.059
30 to 60 min	-0.8	0.80	1.60	0.141

* Mann-Whitney-U-test

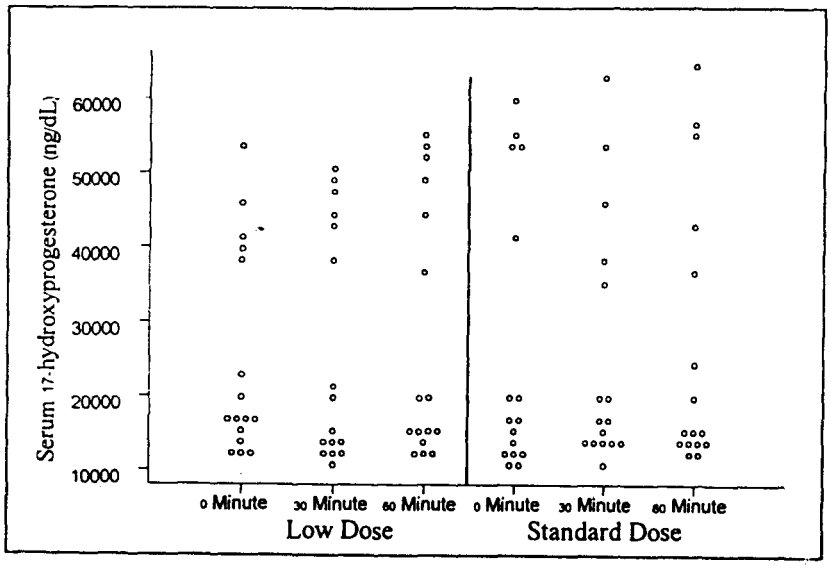

Fig. 2. Comparing serum 17-hydroxyprogesterone (ng/dL) at 0, 30, and 60 minutes between CAH patients on the low *vs* the standard dose ACTH test (8 patients for each group).

Table 4 shows the change in the median serum 17-OHP from one sample time to the other and the differences in the changes. The peak increment for the low dose test occurred between 0 and 30 min; for the standard test, it was between 0 and 60 min, but none were statistically significant.

Using the random effect GEE model for cortisol, the difference in the overall changes between the two groups indicated patients on a low dose of ACTH was 0.06 μg/dl (95% CI: -1.6 to 1.7) higher than those receiving the standard dose, though not statistically significant (p-value = 0.946). The 17-OHP levels in patients on the standard dose of ACTH was 1,725 ng/dl (95% CI: -1,256 to 4,707) higher than those receiving the low dose, again not statistically significant (p-value = 0.257).

DISCUSSION

In normal subjects, the response of cortisol to a rapid ACTH test is an incremental increase of at least 10 μg/dl (276 nmol/L) with the 30 or 60 min value ≥ 20 μg/dl (550 nmol/L)[15,16]. The authors documented no incremental rise in serum cortisol at 30 or 60 min in the low dose test. In the standard test, the peak, and maximal increment, of the cortisol occurred at 60 min and averaged < 10 μg/dl. Baseline cortisol varied in each patient. The baseline level > 20 μg/dl was observed in some CAH patients. The difference between the two groups increased with time, at 60 min it was two-fold greater than the difference at 30 min. Though not statistically significantly different, it indicated that among CAH patients there was no response to the 1 μg dose of ACTH, and

Table 3. Serum 17-hydroxyprogesterone (ng/dl) respone to low (1 μg) and standard dose (250 μg) ACTH test at 0, 30 and 60 min in 16 CAH patients (each group, n = 8) and the differences between the two groups.

Time	Low dose ACTH (Mean ± SD)	Standard ACTH (Mean ± SD)	Differences (Standard-low)	Confidence interval 95%	P-value*
0 min	21,797.2 ± 4,541.7	34,344.5 ± 7,044.9	12,547.3	-5,430.3 to 30,524.9	0.157
30 min	24,519.8 ± 5,706.6	33,821.1 ± 6,640.4	9,301.2	-9,477.6 to 28,080.0	0.306
60 min	25,513.6 ± 6,035.4	36,607.6 ± 7,157.6	11,094.0	-8,986.7 to 31,174.6	0.256

* *t*-test

Table 4. Changes (in median) of serum 17-OHP from 0 to 30 and 60 min, from 30 to 60 min, and the difference of the changes between the low and standard dose ACTH test in CAH patients (each group, n = 8).

Time	Low dose ACTH	Standard dose ACTH	Differences of the Changes (Standard-low)	P-value*
0 to 30 min	441.7	-368.8	-810.5	0.529
0 to 60 min	-676.7	1,788.7	2,465.4	0.036
30 to 60 min	346.5	1,763.9	1,417.4	0.141

*Mann-whitney-U-test

responded poorly to the standard test with limited cortisol increase. The adrenal cortex may have been almost maximally stimulated and unable to increase further cortisol secretion[17]. Both the low and standard ACTH tests demonstrated adrenal insufficiency in all CAH patients.

Normal values in both sexes and all pediatric age groups for 17-OHP were < 500 ng/dl (15 nmol/L) [15]. New et al presented a nomogram of a 60-min ACTH stimulation test in salt-wasting plus simple virilizing of CAH patients, and the range of 17-OHP was between 16,260 and 50,300 ng/dl[1]. In the present study, all 17-OHP levels were > 10,000ng/dl (300 nmol/L). The difference in baseline levels between the two groups were much greater, resulting in peak differences at 0 min (12,547.3 ng/dl) but the average peak levels of both groups occurred at 60 min. For the low dose test, the peak increment was between 0 and 30 min. Five patients had a 17-OHP response < 20,000 ng/dl at 60 min (Fig. 2). In contrast, the peak increment in the standard test occurred between 0 and 60 min and 4 patients had a 17-OHP level > 40,000 ng/dl at 60 min, though this was not a statistically significantly different. Unluhizarci et al studied the comparison between the low and standard dose ACTH test in non-classic CAH with 11-hydroxylase deficiency. The peak cortisol, 17-OHP, and dehydroepiandrosterone sulphate in their study were similar in both tests while 11-deoxycortisol response to the low dose test were significantly lower than the results obtained after the standard test. They showed that the low dose test can not replace the standard test in every clinical situation[18].

The authors were unable to conclude that the 1 μg ACTH test can replace the standard 250 μg test in CAH children because of the unexpected variation in hormones levels. However, the mean and peak responses of the cortisol to both the low and standard dose ACTH tests indicated adrenal insufficiency and the high 17-OHP levels were diagnostic of 21-OH deficiency. If the low dose ACTH test becomes the "standard" test in untreated CAH patients, the diagnosis of 21-OH deficiency would probably not be missed.

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การทำการทดสอบกระตุ้นอะเดรโนคอร์ติโคโทรพิน ในผู้ป่วยต่อมหมวกไตหนาแต่กำเนิด : เปรียบเทียบผลของการใช้ขนาดมาตรฐานและขนาดต่ำ†

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การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบการตอบสนองของต่อมหมวกไตต่อการใช้ยา adrenocorticotropin (ACTH) ขนาดมาตรฐานและขนาดต่ำในผู้ป่วย congenital adrenal hyperplasia (CAH) โดยศึกษาแบบ 2-by-2 crossover study มีผู้ป่วย CAH ที่เข้าร่วมการศึกษาจำนวน 16 คนเป็นชาย 2 คน หญิง 14 คน อายุตั้งแต่ 1.4 เดือนถึง 15 ปี ทุกคนมีอาการสูญเสียเกลือ ผู้ป่วย 8 คน ได้รับการทำ standard dose ACTH (250 μg) test ก่อน อีก 8 คนได้รับการทำ low dose ACTH (1 μg) test ก่อน ทุกคนหยุดยาที่ได้รับก่อนทำการศึกษา 24 ชั่วโมงและตรวจวัดระดับซีรั่ม cortisol และ 17-hydroxy-progesterone (17-OHP) ที่ 0, 30 และ 60 นาที ผลการศึกษาพบว่าซีรั่ม cortisol ก่อนการให้ ACTH ในแต่ละคนมีค่าแตกต่างกันมาก ค่าเฉลี่ยของ cortisol ตอบสนองต่อ standard dose สูงที่สุดที่ 60 นาที ความแตกต่างของซีรั่ม cortisol ระหว่าง 2 กลุ่มที่ 60 นาทีเป็น 2 เท่าของที่ 30 นาที (5.45 และ 2.59 μg/dL) แต่ไม่มีนัยสำคัญทางสถิติ สำหรับค่า 17-OHP ที่ 0 นาทีของทั้ง 2 กลุ่มมีค่าแตกต่างกันมาก ทุกค่าอยู่ในระดับสูงมากกว่า 10,000 ng/dL โดยมีค่าเฉลี่ยที่ 60 นาทีสูงที่สุดทั้ง 2 กลุ่ม ซึ่งการเปลี่ยนแปลงนี้รวมถึงความแตกต่างของระดับ 17-OHP ระหว่าง 2 กลุ่มไม่มีนัยสำคัญทางสถิติ

การศึกษาผลของ low dose และ standard dose ACTH test ต่อซีรั่ม cortisol และ 17-OHP ในผู้ป่วย CAH ยังไม่สามารถสรุปได้ว่าไม่แตกต่างกัน เนื่องจากข้อมูลมี variation ของฮอร์โมนทั้ง 2 ตัวมากกว่าที่คาดมาก แต่จากการศึกษานี้ได้แสดงค่าเฉลี่ยของฮอร์โมนรวมทั้งเวลาที่มีการตอบสนองของฮอร์โมนได้สูงสุดในแต่ละ test โดยทั้ง 2 test บ่งชี้ถึงการขาดเอนไซม์ 21-hydroxylase ในการสังเคราะห์ cortisol จากเปลือกต่อมหมวกไต

คำสำคัญ : โรคเปลือกต่อมหมวกไตหนาแต่กำเนิด, การใช้เอซีทีเอ็ชขนาดต่ำ, การขาดเอ็นซัยม์ 21-ไฮดร็อกซีเลส

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