Long acting porcine sequence ACTH in the diagnosis of adrenal insufficiency

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Abstract

Objectives: Injectable tetracosactide hexa-acetate, ACTH 1-24 (Synacthen), is not marketed in many countries including India, whereas Injectable long acting porcine sequence, ACTH 1-39 (Acton Prolongatum®) is easily available and much cheaper. This study aimed to find the diagnostic accuracy of ACTH stimulation test using i.m. Acton Prolongatum® (acton prolongatum stimulation test, APST) in comparison with Synacthen (short synacthen test, SST) for the diagnosis of glucocorticoid insufficiency.

Methods: Subjects with a suspicion of adrenal insufficiency based on clinical features underwent a SST with 250 µg Synacthen followed by APST using 30 units of Acton Prolongatum®. Serum cortisol levels were measured at 60 and 120 min following injection of Acton Prolongatum®. Stimulated peak cortisol of less than 18 µg/dL on SST was considered as adrenal insufficiency.

Results: Forty seven patients with mean age of 36.7 ± 14.4 years were enrolled for the study. Based on SST, twenty ($n = 20$) persons were classified as having adrenal insufficiency, whereas twenty-seven ($n = 27$) were found to be normal. Area under the curve of APST (at 120 min) was 0.986 when compared to SST, thus proving its high accuracy. A serum cortisol cut off value of 19.5 µg/dL at 120-min following stimulation with Acton Prolongatum® showed a sensitivity of 100% and specificity of 88%.

Conclusion: ACTH stimulation test using Acton Prolongatum® is an economical and accurate alternative to the short Synacthen test.

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Introduction

Early diagnosis of adrenal insufficiency (AI) can be lifesaving (1). Morning cortisol level is useful for the detection of adrenal insufficiency in cases where it is markedly low or clearly in the upper reference range (2, 3). Still, in a majority of patients, stimulation tests are required for the definitive diagnosis of AI. Although the reference test for the diagnosis of AI is measurement of cortisol response to insulin-induced hypoglycemia, it is contraindicated in patients with ischemic heart disease and epilepsy and may be risky in patients with primary AI. Current guidelines suggest the use of standard dose (250 µg) Short Synacthen Test (SST) for the diagnosis of both primary (4) and

secondary AI (5). Synacthen or Cosyntropin (tetracosactide hexaacetate) is a synthetic adrenocorticotrophic hormone (ACTH) composed of the first twenty-four amino acids of the ACTH 1-39 peptide. Synacthen is not marketed in India; hence, the cost is prohibitively high. Constraints of procurement have led to avoidance of ACTH stimulation test even when indicated, with undue reliance on morning cortisol values. Synthetic porcine sequence corticotrophin in carboxymethylcellulose is freely available as Acton Prolongatum® in a 5 mL multidose vial manufactured by Ferring's Pharma. When used at a dose of 30 IU per test it costs 14 times less than 250 µg of Synacthen. Reversible

binding of ACTH to carboxymethyl cellulose protects the peptide against enzymatic breakdown thereby prolonging its action. Acton Prolongatum is preserved at 2–10°C and administered intramuscularly (6).

Although Acton Prolongatum stimulation test (APST) is used in many centers in India for diagnosing AI, there is paucity of literature to strongly support this practice. This study was done to evaluate the performance of APST and validate it against the standard SST.

Subjects and methods

The study was conducted in a prospective observational design, in the Department of Endocrinology, Government Medical College Hospital, Thiruvananthapuram, India. The study was approved by the Human Ethics Committee of Govt. Medical College Thiruvananthapuram and funded by the State Board of Medical Research, Kerala. All patients with clinical suspicion of AI (anorexia, nausea, weight loss, unexplained hyponatremia, progressive hyperpigmentation, history of pituitary macroadenoma or surgery) who attended the outpatient clinic during the study period were included. Written, informed consent was obtained from all the subjects after explaining the procedure. Demographic details and symptomatology of each individual were entered into a pre-designed proforma.

Baseline serum cortisol was measured at between 8:00 h and 9:00 h in all subjects. Short Synacthen Test (SST) was carried out by administering 250 µg of Synacthen intramuscularly to deltoid followed by venous blood sampling for serum cortisol at 30 (SST 30) and 60 (SST 60) min. Three days later APST was performed by injecting 0.5 mL (30 units) of Acton Prolongatum® intramuscularly to deltoid followed by venous blood sampling for serum cortisol at 60 (APST 60) and 120 (APST 120) min. A dose of 30 units was chosen to ensure ease of dispensing (0.5 mL) from a 300 unit in 5 mL formulation. Stimulated peak cortisol cut off of less than 18 µg/dL on SST was considered as AI based on guideline recommendations (4). Patients were closely monitored for any discomfort or adverse events. Delta cortisol was calculated as the maximum increment in serum cortisol following the administration of Acton Prolongatum®. Serum cortisol estimation was done by electrochemiluminescence method using COBAS e 411 analyzer with Cortisol II kits, which has an intra- and inter-assay coefficient of variation of 1.6–2.4% and measuring range of 0.054–63 µg/dL.

Data was tabulated using Microsoft excel and analyzed using SPSS, version 25. Continuous variables

were described using mean and s.d. and categorical variables as frequency and percentages. Receiver-operating characteristic (ROC) curve was plotted using a uniform threshold for any selected variables according to 95% specificity. Area under the curve (AUC) was used to express the overall diagnostic accuracy of the index criterion. *P* value was considered to be significant at 5% level of significance for all comparisons. Youden index was used to find out the cut off levels at which the best possible sensitivity and specificity was obtained.

Results

During the study period, 54 patients with clinically suspected AI were encountered in the outpatient clinic, of whom four were excluded due to liver disease. Thus, 50 persons were included to undergo the SST and APST. The SST was done in 50 patients, of whom three patients did not turn up for the second test and were considered as having withdrawn consent. The remaining 47 patients were included in the final analysis (Fig. 1). The mean age of the study population was 36.7 (±14.46) years. There were 26 (55.3%) female patients and 21 (44.6%) male patients. The mean BMI of the study group was 25.3 ± 5.2 kg/m². None of the patients experienced any immediate adverse events like nausea, vomiting, rash or anaphylaxis with Synacthen or Acton Prolongatum®.

Of the 47 patients with suspected AI, 20 (42.5%) were confirmed to have AI based on the SST using a stimulated peak cortisol cut off of less than 18 µg/dL. Of these, four patients had primary AI and the remaining had secondary AI based on clinical features and ACTH levels.

If 14.5 µg/dL is taken as the cut off for diagnosis of adrenal insufficiency on a SST, as suggested by recent studies which had used the same equipment and kits (Roche COBAS e 411 and Cortisol II), only 16 patients would be classified as having the disease (7, 8, 9).

Performance of APST in subjects with normal adrenal reserve

Among the patients without AI based on SST, the mean basal serum cortisol levels were 7.2 (±1.8) µg/dL and the values at 30 and 60 min were 20.8 (±3.2) µg/dL and 20.3 (±4.0) µg/dL respectively after Synacthen administration. The mean serum cortisol values with APST were 22.6 (±5.1) and 26.1 (±5.5) µg/dL at 60 and 120 min, respectively. The mean serum cortisol values at 1 h after stimulation with Acton Prolongatum® were numerically higher

Figure 1

Flow of the study.

by approximately 2.3 µg/dL when compared to that after Synacthen, but the difference was not statistically significant (Table 1) ($P=0.072$; 95% CI, -4.65 to 0.21).

Performance of APST in subjects with AI

Among the patients with AI, the mean basal serum cortisol levels were 4.5 ± 3.5 µg/dL. During the SST the cortisol levels had a rise to a mean of $7.9 (\pm 5.1)$ µg/dL at 30 min and $9.3 (\pm 6.2)$ µg/dL at 60 min. The mean serum cortisol values

with APST were $9.8 (\pm 6.0)$ µg/dL at 60 min and $10.6 (\pm 6.4)$ µg/dL at 120 min respectively. S. cortisol values at 1 h after stimulation with Synacthen and Acton Prolongatum® were similar ($P=0.796$; 95% CI -4.44 to 3.42) (Table 1).

Receiver-operating characteristic

Receiver-operating characteristic (ROC) analysis was performed for the basal as well as post APST serum cortisol levels (at 60 and 120 min) and area under the curve (AUC)

Table 1 Comparison of cortisol values at different time points during APST and SST in patients with and without AI.

Time (min)	Patients without AI		Patients with AI	
	SST cortisol (µg/dL)	APST cortisol (µg/dL)	SST cortisol (µg/dL)	APST cortisol (µg/dL)
0	7.3 ± 1.9	7.3 ± 1.9	4.6 ± 3.5	4.6 ± 3.5
30	20.9 ± 3.2	NA	7.8 ± 5.0	NA
60	20.3 ± 4.1	22.6 ± 5.2	9.3 ± 6.2	9.8 ± 6.1
120	NA	26.1 ± 5.6	NA	10.6 ± 6.37

Figure 2

Receiver-operator characteristic (ROC) curve plot analysis for morning basal cortisol, APST 60, APST 120 and delta cortisol. The maximum area under ROC was for APST 120 (0.986).

was found out for basal cortisol, APST 60 min, APST 120 min, peak APST cortisol value, mean of post APST cortisol values and Delta cortisol (difference between basal and peak cortisol). Acton Prolongatum® stimulated serum cortisol at 120 min (APST 120) showed maximum area under the curve among the analyzed parameters (Fig. 2 and Table 2). A diagnostic cut off of 19.5 µg/dL provided the highest sensitivity and specificity both for APST 120 (100 and 88% respectively) and peak cortisol after APST (95 and 89%). For the proposed lower (assay specific) SST cut off of 14.5 µg/dL the corresponding cut-off for APST was found to be 18.6 µg/dL both for APST 120 (sensitivity 100% and specificity 84%) and peak cortisol after APST (sensitivity 94% and specificity 84%) based on ROC analysis.

Performance of morning cortisol

Morning cortisol values in patients with AI and those without AI were 7.3 ± 1.9 µg/dL and 4.6 ± 3.5 µg/dL respectively. Although there was a considerable overlap between these values, a morning cortisol level below 3.6 µg/dL provided 100% specificity for diagnosing AI (Table 2). If this value of morning cortisol is taken as a cut off for diagnosis of AI, ACTH stimulation test could have been avoided in 9 (19.1%) out of the 47 patients.

Primary versus secondary AI

Patients with AI were classified as having primary or secondary AI on the basis of clinical features and plasma ACTH levels. Of the 20 patients with AI, 16 had secondary AI and 4 had primary AI. Mean age of patients with primary and secondary AI was 44.5 (19–64) years and 38 (17–80) years and mean BMI was 24.0 kg/m² vs 26.2 kg/m² respectively. Autoimmune adrenalitis and tuberculosis were the probable cause in patients with primary AI. Pituitary surgery, pituitary macroadenoma, stalk interruption syndrome and prior long-term steroid exposure were the etiologies in secondary AI. The mean basal cortisol values were $2.2 (\pm 2.9)$ and $4.8 (\pm 2.9)$ in primary and secondary AI respectively ($P=0.135$). Patients with primary AI achieved a much lower mean peak cortisol level $2.1 (\pm 2.4)$ µg/dL after APST compared to those with secondary AI who had a mean peak cortisol level of $12.7 (\pm 5.1)$ µg/dL.

Discussion

SST is the current standard of care for the diagnosis of AI. Unavailability of Synacthen in large parts of the world makes the diagnosis of AI fraught with challenges of under diagnosis as well as over diagnosis. Synthetic porcine sequence corticotrophin (Acton Prolongatum®) is readily available, stable and cheaper than Synacthen. Present study was done to assess the performance of APST against SST.

Animal studies comparing porcine ACTH in gelatin (1–39 amino acid sequence) and synthetic ACTH (1–24 amino acid sequence) in female dogs revealed no significant difference and concluded that either method caused maximal secretion of the adrenocortical reserve when administered in equimolar amounts (10). Friedman *et al.* studied 11-hydroxycorticosteroid levels following long acting corticotrophin carboxymethyl cellulose and found peak cortisol levels around 1 to 3 h (25.7 µg/dL at 1 h and 37.4 µg/dL at 3 h) (11). Hence, in the present study, serum cortisol values up to 2 h following administration of Acton Prolongatum® were measured. All patients underwent SST followed by APST, where SST was used to classify the patients to be glucocorticoid sufficient or insufficient, and results of APST were compared. It was demonstrated that APST can be safely and reliably utilized for the diagnosis of glucocorticoid insufficiency with a high sensitivity (100%) and specificity (88%). The best cut off for stimulated cortisol after Acton Prolongatum® administration was determined to be 19.5 µg/dL.

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Table 2 Comparison of performance among various parameters obtained from APST.

Test	AUC	S.E.	Best cutoff (sensitivity %, specificity %) ($\mu\text{g/dL}$)	Cutoff for 100% sensitivity ($\mu\text{g/dL}$)	Cutoff for 100% specificity ($\mu\text{g/dL}$)
Basal cortisol	0.775	0.080	5.9 (73, 80)	NA	3.6
APST 60	0.956	0.027	17.0 (95, 88)	20.3	14.9
APST 120	0.986	0.012	19.5 (100, 88)	19.5	17
Peak cortisol after APST	0.978	0.017	19.5 (95, 89)	20.5	16.7
Mean of APST cortisol	0.977	0.017	18.8 (100, 85)	18.8	16.5
Delta cortisol with APST	0.966	0.024	10.8 (84, 100)	15	10.8

(corresponding to an SST cut off of 18 $\mu\text{g/dL}$). Thirty unit dose of Acton Prolongatum[®] was found to be as potent as Synacthen 250 μg in stimulating cortisol secretion in normal as well as glucocorticoid-insufficient patients.

The values of ACTH stimulated cortisol are assay dependent. The proposed newer cut offs for SST while using assays with better sensitivity and analyte specificity (as in the present study) range from 12.7 to 14.5 $\mu\text{g/dL}$ which would be more relevant in patients with secondary AI (7, 8, 9). If a cut off value of 14.5 $\mu\text{g/dL}$ on SST was used for the diagnosis of AI in this study, the corresponding value for APST would be 18.6 $\mu\text{g/dL}$ based on ROC analysis.

In practice, clinical features and plasma ACTH are used to differentiate between primary and secondary AI. In the present study it was noted that patients with primary AI achieve a much lower mean peak cortisol level than those with secondary AI. Further studies may be required to validate APST for the differentiation of primary and secondary adrenal insufficiency as this may be helpful in a setting where plasma ACTH testing is not feasible.

Gunduruthy *et al.* studied the efficacy of porcine sequence corticotropin in healthy volunteers and those with AI albeit without a documented comparison to a gold standard test (ITT or SST) (6). The average cortisol level

achieved in patients with normal adrenal reserve in our study on APST was 26.1 ± 5.6 $\mu\text{g/dL}$, whereas that in the control group of the aforementioned study was 29.6 ± 5.9 $\mu\text{g/dL}$. Another study by Wagmode *et al.* documented the response of both tests but, the study included healthy volunteers only (12). Sharma *et al.* have compared the long acting ACTH (Acton Prolongatum[®]) test against ITT for the diagnosis of secondary AI in children in which the peak values of cortisol obtained on the ACTH test were much higher than those on the ITT (13). This effect was also observed with the SST in several previous studies and is probably related to the supra-physiological plasma levels of ACTH attained with 250 μg of Synacthen which is same as in the case of using Acton-Prolongatum[®] (14). However, the present study, as well as that by Wagmode *et al.* have noted insignificant differences in the stimulated cortisol values with both Synacthen and Acton Prolongatum[®]. The Porcine sequence corticotrophin has also been used to identify AI in critically ill patients and in patients with HIV infection from two different centers in India (15, 16). The findings of previous studies related to Acton Prolongatum[®] are summarized in Table 3.

Using Acton Prolongatum[®] for diagnosis of adrenal insufficiency brings down the cost of investigation

Table 3 Summary of previous studies on porcine ACTH.

Reference	Study subjects	Comparative standard used	Dose of porcine ACTH	Findings
(10)	10 healthy volunteers	nil	40IU	11-hydroxyl corticosteroid peak between 1 to 3 h
(11)	15 female dogs	Synacthen 250 μg	2.2 IU/kg	Similar maximal secretion from adrenocortical reserve by both agents
(6)	24 healthy controls; 24 adrenal insufficient	nil	25 IU	Statistically significant difference in Acton Prolongatum stimulated cortisol and delta cortisol in normal and adrenal insufficient groups
(12)	15 healthy volunteers	Synacthen 250 μg	25 IU	Comparable peak serum cortisol in tetracosactide and porcine sequence corticotropin group ($P = 0.686$)
(13)	48 suspected patients; 28 sufficient, 20 insufficient children	Insulin Tolerance Test	25 IU	Peak cortisol value <18 $\mu\text{g/dL}$ on the IM-ACTH suggestive of AI; Serum cortisol value >22 $\mu\text{g/dL}$ ruled out secondary AI in children (5–14 years)

considerably. Acton Prolongatum® when used at a dose of 30 units per test costs 14 times less than the 250 µg of Synacthen and using APST in lieu of SST can bring down the total cost of diagnosing glucocorticoid deficiency from USD 44 to 11 (Indian Rupees 3100 to 800).

The strength of the present study is the comparison of APST with an existing standard of care (SST), in individuals with both glucocorticoid sufficiency as well as deficiency, being the first one to do so. Limitations of our study include absence of dose titration of porcine ACTH and lack of an assay specific cut-off for Synacthen-stimulated serum cortisol in our laboratory. As opposed to previous studies using 25 units of Acton Prolongatum® for the APST, this study used 30 units considering the ease of dispensing from a 5 mL vial containing 300 units (6, 12, 13). No adverse events were observed with Synacthen or Acton Prolongatum in the present study. Anaphylactic reactions to either can occur, although rarely in the present era due to the improved methods of purification (17).

Conclusion

Acton Prolongatum® stimulation test (APST) can accurately diagnose glucocorticoid insufficiency. Use of this long-acting porcine sequence ACTH in conjunction with morning cortisol levels can bring down the cost of diagnosing glucocorticoid insufficiency considerably and make the test accessible and affordable to large parts of the world.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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