COMPARISON OF LOW DOSE SHORT SYNACTHEN TEST (1 MICROGRAM) WITH STANDARD DOSE SHORT SYNACTHEN TEST (250 MICROGRAM) IN PATIENTS OF ADRENAL INSUFFICIENCY

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Abstract

Objective: To determine the diagnostic accuracy of low dose 1µg short synacthen test taking standard dose 250µg short synacthen test as gold standard.
Study design: A descriptive study.
Place and Duration of study: Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology Rawalpindi from Jan 2006 to Jan 2007.
Patients and Methods: Thirty patients with clinical suspicion of adrenal insufficiency and equal number of age matched healthy males and females as controls were included in the study. Relevant clinical history and physical examination was recorded on designated proforma. Short synacthen test was performed between 0800 – 1000 h by using ACTH doses of 1µg and 250 µg with interval of 3 days in all patients and controls. Three blood samples were obtained for cortisol (basal, 30 min and 60 min after I/M ACTH injection).
Results: Using 250µg short synacthen test as a standard test, the 1µg short synacthen test had sensitivity of 100 %, specificity of 72%, positive predictive value of 71 % and negative predictive value of 100 % and 83% accuracy.
Conclusion: The low dose 1 µg short synacthen test is as sensitive as standard dose 250 µg short synacthen test but less specific in the diagnosis of adrenal insufficiency.

Keywords : Addison's disease, sensitivity, specificity, positive and negative predictive values

Article

INTRODUCTION

Adrenal insufficiency is an uncommon clinical disorder that results from an inadequate basal or stress level of plasma cortisol1. The presentation of adrenal insufficiency may be insidious and thus difficult to recognize. It is important to diagnose adrenal insufficiency because the disorder may be fatal if left unrecognized or untreated2. With diagnosis and appropriate adrenocortical hormone replacement, normal quality of life and longevity can be achieved2. Once suspected, the definitive diagnosis can be confirmed by laboratory evaluation of adrenocortical function by short synacthen test, insulin tolerance test and metyrapone test. The established reference test for the assessment of the hypothalamo-pitutary adrenal axis is to measure the cortisol response to insulin induced hypoglycemia3.This test is unpleasant for the patient, potentially dangerous, resource intensive and contraindicated in patients with ischemic heart disease and epilepsy4. The short synacthen test is widely used to evaluate functional reserve of adrenals and is easy to perform. A highly significant correlation between short synacthen test and insulin tolerance test (ITT) has been a consistent finding in the literature5-7. The short synacthen test can be used as the initial test used to evaluate patients for both primary and secondary adrenal insufficiency.

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Received: 31 Dec 2008; **Accepted:** 19 Oct 2009

The standard dose short synacthen test utilizes 250 µg ACTH dose to observe the cortisol response of the adrenal cortex. However, several study groups have reduced this dose considerably and demonstrated that the cortisol response to 1µg ACTH is equivalent to that obtained with 250 µg

ACTH in normal subjects8,9. It seems logical to assume that the supraphysiological dose of 250µg used in the standard dose test might over stimulate partially atrophied adrenals and produce a deceivingly adequate cortisol response. The 1µg ACTH has the advantage of cost effectiveness as well as safety, as it avoids subjecting the patient to undue high doses of ACTH as happens in standard dose synacthen test. Therefore, this study was planned to evaluate efficacy of 1µg ACTH dose.

PATIENTS AND METHODS

This descriptive study was conducted at the Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology Rawalpindi from Jan 2006 to Jan 2007. All those cases who presented at endocrine clinic of AFIP with clinical suspicion of adrenal insufficiency such as generalized weakness, fatigue, hyperpigmentation of skin and mucous membranes, weight loss and hypotension were included. Apparently healthy males and females of the similar age groups with normal BMI were included in study as controls.

Diagnosed cases of Addison's disease on treatment, patients under treatment due to other endocrine disorders i.e. thyroid disorders and pregnant subjects were excluded from the study. Short synacthen test was performed between 0800 – 1000hrs. An indwelling venous cannula was placed into one forearm and sample for basal cortisol was taken. All the subjects and controls were subjected to both 1 µg and 250 µg short synacthen tests. Order of 1 µg and 250 µg short synacthen tests was randomized between subjects and controls. Both tests were done after a gap of 3 days. Two additional samples for serum cortisol were collected after 30 and 60 minutes of ACTH injection. The tubes were properly labelled and the specimens were transported to the processing room within half an hour and allowed to clot at room temperature. Serum was then separated by centrifugation at a relative centrifugal force of 2000-3000 g for about 15 minutes. Serum was frozen for estimation of cortisol in batches. Serum cortisol was analyzed by chemiluminescence method on immulite 2000 analyzer.

RESULTS

In this study, thirty suspected Addison's disease/cases were investigated in which 14 (47%) were males and 16 (53%) females. Age ranged from 2 to 62 years with mean age of 32 years. Weight ranges from 7 to 75 kg with mean weight 41kg (Table-1).

Table-1: Increment of cortisol from baseline after 250 microgram ACTH stimulation test and 1 microgram ACTH stimulation in suspected Addison's disease patients

	Basal cortisol mean (SD)	Cortisol after 30 min mean (SD)	Cortisol after 60 min mean (SD)
250 microgram ACTH stimulation test	202 (67)	349 (86)*	419 (96)*
1 microgram ACTH stimulation test	210(70)	332(72)*	293 (40)

p value is significant at less than 0.05 as compared to baseline

In suspected Addison's disease cases the conventional 250 µg ACTH dose caused maximum cortisol increment both at 30min and 60min from baseline ($p < 0.05$) whereas with 1 µg ACTH dose significant cortisol increment was observed only at 30 min ($p < 0.05$) (Table-1).

The control group included 14 males and 16 females. Age ranged from 6 to 62 years with mean age of 34 years. Weight ranged from 22 to 82 kg with mean weight 52kg. In the controls, the standard 250µg ACTH stimulation test caused maximal stimulation of adrenal glands to produce significant cortisol increments both at 30min and 60min from baseline cortisol ($p < 0.05$). Similarly, 1µg ACTH stimulation test also caused maximal adrenal stimulation to produce significant cortisol increments at 30min and 60min from baseline in controls ($p < 0.05$) (Table-2).

Table-2: Increment of cortisol from baseline after 250 microgram ACTH stimulation and 1 microgram ACTH stimulation tests in controls

	Cortisol basal mean (SD)	Cortisol after 30 min mean (SD)	Cortisol after 60 min mean (SD)
250 microgram ACTH stimulation test	349 (53)	682 (69)*	793 (78)*
1 microgram ACTH stimulation test	348 (61)	598 (60)*	502 (59)*

p value is significant at less than 0.05 as compared to baseline

Out of total thirty suspected patients, thirteen patients failed to respond to both low 1µg and 250µg ACTH dose short synacthen tests (true positives). In twelve patients, both low 1µg and 250µg ACTH dose resulted in cortisol increment greater than 200nmol/L (true negatives). Five patients responded to 250µg ACTH dose but failed to respond to 1µg ACTH dose (false positives). No false negative

test results were obtained i.e., individuals who responded to 1µg ACTH dose but failed to respond to 250µg ACTH dose.
Considering 250µg ACTH stimulation test as a standard, sensitivity of low dose 1µg short synacthen test i.e., the percentage of individuals correctly detected was 100% and specificity of low dose 1µg short synacthen test i.e., the percentage of disease free individuals correctly detected was 71% (Fig.1).

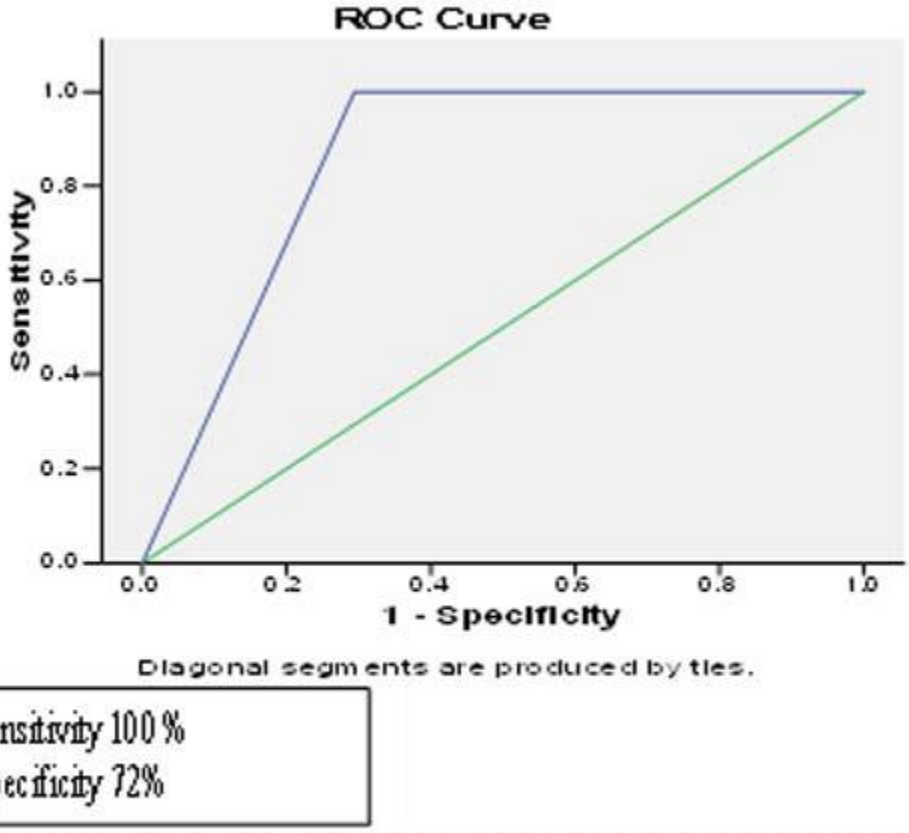

Figure 1: ROC Curve for 1 microgram ACTH test in suspected cases of Addison's disease

Positive predictive value of 1µg short synacthen test i.e., the likelihood that a person with positive result truly had the disease was 72 %. Negative predictive value of 1 µg short synacthen test i.e., the likelihood that a person with negative result truly had no disease was 100 %. Accuracy i.e., the proportion of all tested patients correctly identified by the 1 µg short synacthen test was 83%.

DISCUSSION

The most common problem in diagnosing primary adrenal insufficiency is lack of clinical suspicion as the condition is rare and the signs & symptoms non-specific10. Adrenal insufficiency ranges from subclinical hypoadrenalism to overt primary adrenal insufficiency. Subclinical hypoadrenalism is characterized by a normal cortisol response to ACTH and an elevated basal ACTH level. While primary adrenal insufficiency is characterized by a negligible cortisol response to synthetic ACTH injection11. Despite optimized life-saving glucocorticoids and mineralocorticoids replacement therapy, health-related quality of life in adrenal insufficiency is more severely impaired than previously thought12. Once suspected, the definitive diagnosis can be confirmed by laboratory evaluation of adrenocortical function by short synacthen test, insulin tolerance test and metyrapone test13. The reference test for the assessment of the hypothalamo-pitutary adrenal axis is to measure the cortisol response to insulin induced hypoglycemia but it is an unpleasant, resource intensive test and may cause serious complications in the elderly patients with cardiac disease, epilepsy, or hypothyroidism without treatment and requires close medical supervision13.

It has been suggested that the short synacthen test may be used instead of the insulin tolerance test to evaluate the hypothalamic-pituitary adrenal axis but it has produced some falsely reassuring results with potentially serious consequences14. Like the hypothalamic-pituitary disorders, early diagnosis of the initial or partial stages of primary adrenocortical insufficiency has an important role. The synacthen test can be more sensitive if physiological or near physiological dose of ACTH such as 1 µg is used. The 1 µg ACTH is reported to be more sensitive in cases of mild adrenal insufficiency and allows assessing pituitary adrenal suppression after long term treatment with glucocorticoids15. However, the validity of low dose 1 µg short synacthen test is reported mainly for the assessment of secondary adrenocortical insufficiency16. Although 1µg ACTH is accepted as the lowest physiologic dose in the evaluation of adrenal gland functions, this test is not standardized and a number of problems may emerge in non specialized centers without adequate training of staff in the IV or IM delivery of such a small quantity of the drug. The present study confirms that the administration of 1µg ACTH was able to induce a significant cortisol rise in healthy subjects. In our study, considering 250 µg short synacthen test as gold standard for diagnosis of Addison's disease, 1µg short synacthen test had 100% sensitivity and 72% specificity, positive predictive value of 72%, negative predictive value of 100% and accuracy of 83%.

Various studies confirmed the results of our study such as Dickstein et al9, Tordjman et al15, Weintrob N et al17 by showing that 1 µg short synacthen test resulted in cortisol response greater than 500 nmol/l or increment of 200 nmol from baseline. Ambrosi et al18 confirmed that 1µg ACTH test is a useful, safe and inexpensive tool for the initial assessment of hypothalamo-pitutary adrenal function.

Contreras et al19 concluded that measurement of salivary cortisol in response to 25 microgram of corticotrophin injected into the deltoid muscle may become a useful and relatively noninvasive clinical tool to detect subclinical hypoadrenal states.

After 250µg short synacthen test, peak cortisol response was attained at 60 min in controls and in suspected Addison's disease cases due to sustained response to high dose exogenous ACTH. This finding coincides with the study carried out in Aga Khan University hospital Karachi by Mansoor et al20 who concluded that a 60 min cortisol during 250 µg short synacthen test was reliable enough in identifying normal subjects and to exclude adrenal insufficiency. Whereas, peak cortisol levels were attained at 30 min after 1µg short synacthen test in controls and suspected Addison's disease cases due to short lived response of low dose ACTH. The low dose 1µg short synacthen test has advantages of cost effectiveness as well as safety to the patient for subjecting them to undue high doses of synthetic ACTH.

CONCLUSION

This study concludes that low dose 1 µg short synacthen test is as the sensitive as standard dose 250 µg short synacthen test but is less specific in the diagnosis of adrenal insufficiency

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