

Research Article

Which Serum Cortisol after High Dose Short Synacthen Test, 30 or 60 Minutes?

Abbas Ali Mansour*, Ali Hussein Ali Alhamza, Ammar Mohammed Saeed Abdullah Almomin
Endocrine and Metabolism Division, Al-Faiha Diabetes Endocrine and Metabolism Center (FDEMC), Basrah College of Medicine, Iraq

*Corresponding author: Abbas Ali Mansour, Director of Al-Faiha Diabetes Endocrine and Metabolism Center (FDEMC), Endocrine and Metabolism Division, Basrah College of Medicine, Iraq, Tel: 009647801403706; Email: aambaam@gmail.com

Received: December 13, 2014; Accepted: January 02, 2015; Published: January 06, 2015

Abstract

Background: Short synacthen (cosyntropin) test, has replaced the insulin stress test as the first-line test to assess adrenal insufficiency. The aim of this study was to determine the utility of the 30 and 60 minute cortisol measurement in the high dose (250 µg) short synacthen test.

Methods: Cross sectional study was conducted by reviewing the database of patients underwent short synacthen test in Al-Faiha Diabetes Endocrine and Metabolism Center (FDEMC) for the period from November 2009 to May 2013.

Results: Study participants includes 435 patients. The cortisol response in short synacthen test was sufficient in 198 (45.5%) patients and abnormal in 237 (54.4%) patients. It was insufficient at 30 minute only in 56 (12.9%) patients, insufficient at 60 minute only in 5 (1.1%) patients and insufficient at both 30 minute and 60 minute in 176 (40.5 %) patients. Insufficient at 30 minute and sufficient at 60 minute was seen in 120 (27.6 %) patients. This means that the false negative test if the 60 minute sample was not taken was 27.6 %. Only 5 (1.1%) patients with normal response at 30 minutes will regress to response at 60 minutes.

Conclusion: measuring both 30 minute and 60 minute cortisol level are necessary and at 60 minute is fundamental in interpretation of short synacthen test.

Keywords: Adrenal insufficiency; Cortisol; Short synacthen test

Introduction

Adrenal insufficiency is caused by either primary adrenal failure (mostly due to autoimmune adrenalitis) or by hypothalamic-pituitary impairment of the corticotrophic axis (predominantly due to pituitary disease). It is a rare disease, but is life threatening when overlooked [1].

Symptoms commonly associated with adrenal insufficiency are "fatigue" (lack of energy or stamina), abdominal pain, nausea and dizziness (hypotension symptoms) and all are nonspecific [2].

Although different tests for adrenal insufficiency have been developed, few have been adequately studied and many are inconvenient for use in outpatient clinical settings [3]. Short synacthen (cosyntropin) test (SST) has replaced the insulin stress test as the first-line test to assess adrenal insufficiency and has received considerable attention regarding its sensitivity and specificity [4, 5].

The synthetic polypeptide Synacthen (Tetracosactrin BP) has a structure identical to the N-terminal 24 amino acids of Adrenocorticotrophic Hormone (ACTH). It has a short duration of action and permits a rapid and convenient screening test for the assessment of adrenocortical function by measuring cortisol response. Routinely taking a 60 minute sample would improve the specificity of SST and avoid misdiagnosis of adrenal insufficiency [6]. In view of biological and analytical variation, it is recommended that each laboratory should use their own results validated at the three time points [6]. Half-life for synacthen (cosyntropin) is only 15 minutes. Administration is by intravenous or intramuscular injection

and a rise in cortisol should generally be seen around 30 minutes after administration. Plasma cortisol levels usually peak about 45 to 60 minutes after injection [7]. Comparison of the plasma cortisol response at 30 minutes with both short ACTH tests and the peak in the insulin tolerance test did not reveal differences [8].

The cosyntropin test works well in patients with primary adrenal insufficiency, but the lower sensitivity in patients with secondary adrenal insufficiency [7].

All laboratories in UK stated that a dosage of 250µg synacthen administered during the Synacthen test and most generally agreed that a basal and 30 minute blood sample should be taken, while a significant number analyzed a 60 minute sample [4]. Two UK surveys conducted show an increasing trend in clinicians discarding the 60 minute sampling time and relying more heavily on the 30 minute sample [4-9]. The only time point that has been validated against the insulin tolerance test. Two studies stated that the 60 minute sample has no benefit over the 30 minute sample [10, 11].

The definition of the 'normal' response to synacthen should be both gender and method related at all time points [12].

The aim of this study was to determine the utility of the 30 and 60 minute cortisol measurement in the high dose (250 µg) short synacthen test.

Material and Methods

Cross sectional study was conducted by reviewing the database of patients underwent short synacthen test in Al-Faiha Diabetes

Endocrine and Metabolism Center (FDEMC) for the period from November 2009 to May 2013 in Basrah (Southern Iraq).

Inclusion criteria

Patients referred to Al-Faiha Diabetes Endocrine and Metabolism Center (FDEMC) to assess adrenal function for following reasons.

- Glucocorticosteroids use: Any patient who has received the equivalent of 15mg/day of prednisolone for more than 3 weeks [13].
- Unexplained symptoms: like postural dizziness, hypotension, fatigue, weight loss or pigmentations [1].
- *Addison's disease*: with florid features.

Exclusion criteria

Pregnant women, those on oral contraceptive pills or corticosteroid use in the preceding one week and those with repeated short synacthen test.

Preparation

Short, or rapid, ACTH test, measurement of cortisol in blood is repeated 30 to 60 minutes after an intravenous 250µg ACTH injection (Tetracosactrin 0.25µg, Synacthen®; Ciba-Geigy, France). The patient does not need to be fasted. It was done at 8-10 AM. Blood samples were taken immediately before the test for the determination of basal serum cortisol concentration and at 30 minute and 60 minute thereafter [1].

Serum cortisol was measured with use of electro chemiluminescent (ECL) automated immunoassays applied on cobas e 411 analyzer (Roche) using 2nd generation platform of ECL technology at 0, 30 and 60 minute after intravenous or intramuscular synacthen (250 µg).

Interpretation of results

Normal response: Post-stimulation serum cortisol should be greater than 20 ug/dL (555 nmol/L) [4].

Statistical analysis

Statistical analysis was performed using a computer program package (SPSS 15 for Windows, Standard version, ©SPSS. Inc, USA) and a P value of <0.05 was considered significant.

Results

Study participants (Table 1) include 435 patients with mean age of 34.78 ± 11.85 year and age range 12-78 year. Of them women constitutes 64.6% with mean BMI of 25.36 ± 6.18 kg/m². The mean basal cortisol in ug/dL was 9.68 ± 8.14 , 30 minute cortisol 18.17 ± 11.03 and 60 minute cortisol was 21.41 ± 12.61 .

The commonest indication of short synacthen test (Table 2) in this center was previous glucocorticosteroids use in 62.8% followed by unexplained symptoms in 33.6% and florid features of *Addison's disease* in only 3.4%.

The cortisol response in short synacthen test (Table 3) was sufficient in 198 (45.5%) patients, and abnormal in 237(54.4%) patients. It was insufficient at 30 minute only in 56 (12.9%) patients, insufficient at 60 minute only in 5 (1.1%) patients and insufficient at both 30 minute and 60 minute in 176 (40.5 %) patients. Insufficient at 30 minute and sufficient at 60 minute was seen in 120(27.6 %) patients.

Table 1: Study baseline.

Age (years)	Mean $\pm$ SD	34.78 $\pm$ 11.85
	Range	12-78
Gender N(%)	Men	154(35.4)
	women	281 (64.6)
BMI (kg/m2) Mean $\pm$ SD		25.36 $\pm$ 6.18
Serum cortisol µg/dL (Mean $\pm$ SD)	Basal	9.68 $\pm$ 8.14
	30 minute	18.17 $\pm$ 11.03
	60 minute	21.41 $\pm$ 12.61
Total N(%)		435(100.0)

Table 2: Indication of short synacthen test among 435 patients.

	N(%)
Glucocorticosteroid use	273(62.8)
Unexplained symptoms	146(33.6)
Addison's disease	15(3.4)
Cushing syndrome due to adrenal adenoma after surgery	1(0.2)
Total	435(100.0)

Table 3: Sufficiency of short synacthen test.

	N (%)
Sufficient at both 30 minute and 60 minute	198(45.5)
Insufficient at 30 minute only	56 (12.9)
Insufficient at 60 minute only	5 (1.1)
Insufficient at both 30 minute and 60 minute	176 (40.5)
Insufficient at 30 minute and sufficient at 60 minute	120(27.6)

Table 4: Predictors of abnormal response.

	N (%)	95% confidence interval (CI)	Odd ratio	P value
Age >30 year	174(40)	1.301-2.830	1.919	<0.001
Women	281 (64.6)	0.644-1.420	0.956	0.841
BMI $\geq$ 25 (kg/m2)	235(54.0)	0.888-1.895	1.297	0.209
Glucocorticosteroid use	273(62.8)	0.816-1.786	1.207	0.371
Unexplained symptoms	146(33.6)	0.556- 1.240	0.830	0.415

This means that the false negative test if the 60 minute sample was not taken was 27.6 %.Only 5 (1.1%) patients with normal response at 30 minutes will regress to response at 60 minutes.

On univariate analysis (Table 4) of factors associated with adrenal insufficiency, only age >30 year was associated (OR 1.919, 95% CI [1.301; 2.830], p-value <0.001). The women gender, BMI, glucocorticosteroids use, or unexplained symptoms were not associated with abnormal response.

Discussion

The current study was undertaken to evaluate utility of the 30 and 60 minute cortisol measurement in the high dose (250 µg)short synacthen test. In this study insufficient response at both 30 minute and 60 minute was seen in 40.5% and 5 (1.1%) patients had insufficient response at 60 minute alone. In Karachi ,Pakistan ,the short synacthen test showed abnormal response in 25% at both 30- and 60-minute values and only one (0.4%) had insufficient at 60

minute [14]. The authors concluded that 30 minutes and 60 minutes cortisol value are equally effective in identifying abnormal cases and that 60 minutes cortisol level will be cost effective in low resource countries with similar finding also reported Alesci et al [15].

In our study only 5 (1.1%) patients with normal response at 30 minutes will regress to response at 60 minutes. This was not seen in 2 large urban National Health Teaching Hospitals in the UK, where no individuals had sufficient response at 30 minute and then failed to respond at 60 minute [6].

We found that measuring cortisol at 30 minutes alone will miss 27.6 % of those with adrenal insufficiency (false negative). In National Health Teaching Hospitals in the UK, significant proportion of people (11%) undergoing short synacthen tests may be inappropriately diagnosed as having adrenal insufficiency if a 30-minute cut off of 550nmol/L is used, and that why they suggested a 60 minute sample is required to improve the specificity of the short synacthen tests [6].

In that study they use of the 60 min sample alongside the 0 and 30 minute samples in the conventional short Synacthen tests to prevent unnecessary over-diagnosis of adrenal insufficiency. Despite that insufficient response at 30 minute only was seen in 56 (12.9%) patients in this study, the 30 minute cortisol level again cannot be ignored also.

This mean that although the 60 minute sample of cortisol was not validated against gold standard insulin tolerance test, it seems to be very important in interpretation of short synacthen test and deleting sampling at this time will affect the results of test greatly.

Alesci et al found that after intramuscular administration of 250µg ACTH, followed by a single cortisol measurement at 60 min, is comparable to 24hour cortisol sampling and represents a valid and rapid screening test of adrenal function in healthy men [15].

Nonprescription glucocorticosteroids use among patients attending the outpatient in Basrah was seen in 2.6%, of them 15.3% presented with acute adrenal failure [16]. But in our study, glucocorticosteroids use was not predictor of adrenal insufficiency, in contrary to study from Switzerland were a history of glucocorticosteroids withdrawal within weeks or months prior to the hospitalization was highly suggestive of secondary adrenal failure in 281 in-patients [17].

Conclusion

Measuring both 30 minute and 60 minute cortisol level are necessary and at 60 minute is fundamental in interpretation of short synacthen test.

Acknowledgements

The authors would like to acknowledge all the medical staff of Al-Faiha Diabetes Endocrine and Metabolism Center (FDEMC).

References

1. Arlt W, Allolio B. Adrenal insufficiency. *Lancet*. 2003; 361: 1881-1893.
2. Neary N, Nieman L. Adrenal insufficiency: etiology, diagnosis and treatment. *Curr Opin Endocrinol Diabetes Obes*. 2010; 17: 217-223.
3. Dorin RI, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. *Ann Intern Med*. 2003; 139: 194-204.
4. Chatha KK, Middle JG, Kilpatrick ES. National UK audit of the short synacthen test. *Ann Clin Biochem*. 2010; 47: 158-164.
5. Agha A, Tomlinson JW, Clark PM, Holder G, Stewart PM. The long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic-pituitary-adrenal axis. *J Clin Endocrinol Metab*. 2006; 91: 43-47.
6. Chitale A, Musonda P, McGregor AM, Dhataria KK. Determining the utility of the 60 min cortisol measurement in the short synacthen test. *Clin Endocrinol (Oxf)*. 2013; 79: 14-19.
7. Hamilton DD, Cotton BA. Cosyntropin as a diagnostic agent in the screening of patients for adrenocortical insufficiency. *Clin Pharmacol*. 2010; 2: 77-82. doi: 10.2147/CPAA.S6475.
8. González J, Villabona C, Ramón J, Navarro MA, Giménez O, Ricart W, et al. Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in normal subjects. *Clin Endocrinol (Oxf)*. 2000 Aug; 53: 199-204.
9. Barth JH, Seth J, Howlett TA, Freedman DB. A survey of endocrine function testing by clinical biochemistry laboratories in the UK. *Ann Clin Biochem*. 1995; 32: 442-449.
10. Gleeson HK, Walker BR, Seckl JR, Padfield PL. Ten years on: Safety of short synacthen tests in assessing adrenocorticotropin deficiency in clinical practice. *J Clin Endocrinol Metab*. 2003; 88: 2106-2111.
11. Hurel SJ, Thompson CJ, Watson MJ, Harris MM, Baylis PH, Kendall-Taylor P. The short Synacthen and insulin stress tests in the assessment of the hypothalamic-pituitary-adrenal axis. *Clin Endocrinol (Oxf)*. 1996; 44: 141-146.
12. Clark PM, Neylon I, Raggatt PR, Sheppard MC, Stewart PM. Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. *Clin Endocrinol (Oxf)*. 1998; 49: 287-292.
13. Jung C, Inder WJ. Management of adrenal insufficiency during the stress of medical illness and surgery. *Med J Aust*. 2008; 188: 409-413.
14. Mansoor S, Islam N, Siddiqui I, Jabbar A. Sixty-minute post-Synacthen serum cortisol level: a reliable and cost-effective screening test for excluding adrenal insufficiency compared to the conventional short Synacthen test. *Singapore Med J*. 2007; 48: 519-523.
15. Alesci S, Ilias I, Souvatzoglou E, Harris AG, Gold PW, Ayala AR, et al. Intramuscular administration of ACTH1-24 vs. 24-hour blood sampling in the assessment of adrenocortical function. *Hormones (Athens)*. 2005; 4: 96-100.
16. Mansour AA, Odaa AH, Wanoose HL. Corticosteroid nonprescription use: a cross-sectional hospital-based study in Basrah. *Med Princ Pract*. 2010; 19: 182-187.
17. Oboni JB, Marques-Vidal P, Pralong F, Waeber G. Predictive factors of adrenal insufficiency in patients admitted to acute medical wards: a case control study. *BMC Endocr Disord*. 2013; 13: 3.