

# 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

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## ABSTRACT

**Introduction:** Previous studies have indicated that most individuals reach peak cortisol levels in 60 minutes during the short Synacthen test (SST) done to exclude adrenal insufficiency. Therefore, measuring serum cortisol at only 60 minutes may suffice. This study was carried out to evaluate the significance of the 60-minute serum cortisol level in SST as a reliable and cost-effective screening test in comparison to the conventional SST.

**Methods:** A cross-sectional study was conducted from January 2000 to September 2004, in which data was collected by reviewing medical records of all patients who underwent SST at the Aga Khan University Hospital, Karachi. A total of 236 patients suspected of having adrenal insufficiency were included. Values of serum cortisol at baseline, 30 and 60 minutes post-250 ug-injection Synacthen were recorded. The cortisol level was measured through fluorescence polarisation immunoassay. The cut-off value of 20 ug/dL was used to differentiate normal individuals from hypoadrenal individuals.

**Results:** Out of 236 study participants, 93 (39 percent) were males and 143 (61 percent) were females. The mean age and standard deviation was 44.4 +/- 21 years. Cortisol concentration increased significantly from baseline to 30 minutes and 60 minutes after injecting Synacthen (p-value is less than 0.001 for each). The majority of the patients reached the cortisol peak of greater than 20 ug/dL (555 nmol/L) at 60 minutes. Normal responses were found in 148 patients (63.1

percent) at both 30 and 60 minutes, while 27 participants (12 percent) reached a peak greater than 20 ug/dL (555 nmol/L) at 60 minutes but were less than 20 ug/dL (555 nmol/L) at 30 minutes. In the deficient cases, SST showed abnormal responses in 60 cases (25 percent) at both 30 and 60 minutes. However, there was only one patient who reached a peak value of 21 ug/dL (589 nmol/L) at 30 minutes, which reduced to 17 ug/dL (485 nmol/L) at 60 minutes.

**Conclusion:** This study showed that a 60-minute cortisol value during SST was reliable enough in identifying normal subjects for excluding adrenal insufficiency, and was equally effective in identifying abnormal cases, as compared to values at both 30 and 60 minutes. It is therefore suggested that a single 60-minute post-Synacthen serum cortisol level may suffice, as compared to the conventional SST. This is also significant as a cost-effective measure, especially in third world countries where cost is a major issue for diagnosing and treating patients.

**Keywords:** adrenal insufficiency, cortisol, short Synacthen test, Synacthen

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## INTRODUCTION

Adrenal insufficiency (AI) is an uncommon clinical disorder, which results from an inadequate basal or stress level of plasma cortisol. It is important to diagnose adrenal insufficiency because the disorder may be fatal if left unrecognised or untreated.<sup>(1)</sup> With diagnosis, and appropriate adrenocortical hormone replacement, normal quality of life and longevity can be achieved. The presentation of adrenal insufficiency may be insidious and thus difficult to recognise. Once suspected, however,

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a definitive diagnosis can be confirmed through laboratory evaluation of adrenocortical function.<sup>(1)</sup>

Although different tests for AI have been developed, few have been adequately studied and many are inconvenient for use in outpatient clinical settings.<sup>(1)</sup> The plasma cortisol level obtained in the basal condition usually does not provide useful information on the glucocorticoid status of an individual, unless the levels are too high or too low, which obviates the need for further testing.<sup>(2)</sup> Measurement of maximal cortisol concentration during short Synacthen test (SST), however, is an excellent method to exclude any abnormality of adrenal function.<sup>(2-6)</sup> Several dynamic tests such as, insulin tolerance test (ITT) and metyrapone test are also frequently used clinically.<sup>(7-10)</sup> However, these tests also have limitations because of the risk of hypoglycaemia or the risk of acute adrenal crisis.<sup>(11,12)</sup>

The most convenient and least risky test is SST.<sup>(13-16)</sup> It is, therefore, widely used in many different clinical settings and is easy to perform. In addition, data on performance of SST in various clinical settings is plentiful. The standardised initial test commonly used to evaluate patients for both primary and secondary AI is SST.<sup>(1)</sup> In Longui et al's study on 64 patients,<sup>(2)</sup> during SST, the serum cortisol measurement in most of the patients reached the maximum response of  $21.2 \pm 2.5$   $\mu\text{g/dL}$  ( $587 \pm 69$  nmol/L) at 60 minutes. This was also reported by other studies.<sup>(17,18)</sup> This signifies the point that measuring 60-minute serum cortisol in SST may suffice for evaluating adrenal sufficiency. The principle objective of this study was to evaluate the use of plasma cortisol at 60-minute post-Synacthen injection, instead of measuring cortisol at baseline, 30 minutes and 60 minutes, as is currently done in conventional SST. This strategy will reduce the number of cortisol measurements from three to one in the SST, thus making it more cost-effective and convenient than the conventional SST.

## METHODS

In this cross-sectional study, data obtained from medical records of 236 patients, who had been subjected to the SST, were included. Values of serum cortisol at baseline, 30 and 60 minutes, post-250  $\mu\text{g}$  Synacthen injection, were recorded. All age groups were included. These patients were administered one dose of 250  $\mu\text{g}$  tetracosactide (synthetic ACTH 1-24, Synacthen, Ciba Giegy, Basel, Switzerland) each. 25 patients were administered this dose through intramuscular, and 213 through intravenous, Synacthen injection. This cross-sectional study lasted for approximately five years from January 2000 to September 2004. Medical records of all the patients who underwent SST at the Aga Khan University (AKU) Hospital, Karachi, were reviewed.

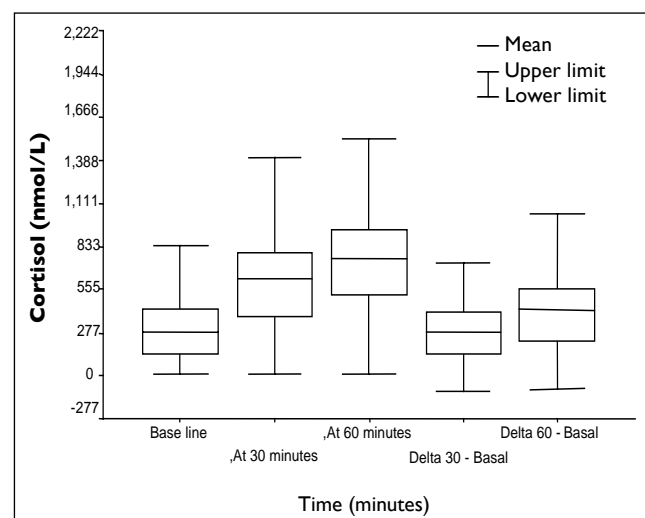
Plasma cortisol levels were determined at baseline,

30 and 60 minutes post-Synacthen injection, after separation by centrifugation in the Chemical Pathology Section of the clinical laboratory at the AKU. The cortisol level was measured using the fluorescence polarisation immunoassay (FPIA) (Abbott Laboratories, Diagnostic Division, Abbott Park, USA). To differentiate between normal or abnormal subjects, post-Synacthen cortisol level at 30 minutes, 60 minutes or both, that was greater than or equal to 20  $\mu\text{g/dL}$  (555 nmol/L) was classified as normal (non-diseased), while a level of less than 20  $\mu\text{g/dL}$  (555 nmol/L) was classified as sub/abnormal (diseased).<sup>(7)</sup>

Statistical analysis of data was performed by using the Statistical Package for Social Sciences version 11.5.0 (SPSS Inc, Chicago, IL, USA). Descriptive analysis was done for demographical and laboratory tests. Results are presented as mean  $\pm$  standard deviation for quantitative variables, and as number (percentage) for qualitative variables. Repeated measured analysis of variance was used to assess the mean difference of plasma cortisol level at baseline to 30 and 60 minutes, following intramuscular and intravenous Synacthen injection. Correlation among plasma cortisol levels at 30 and 60 minutes was estimated using the Pearson coefficient of correlation ( $r$ ). A  $p$ -value of less than 0.05 was considered as statistically significant. All  $p$ -values were two-sided.

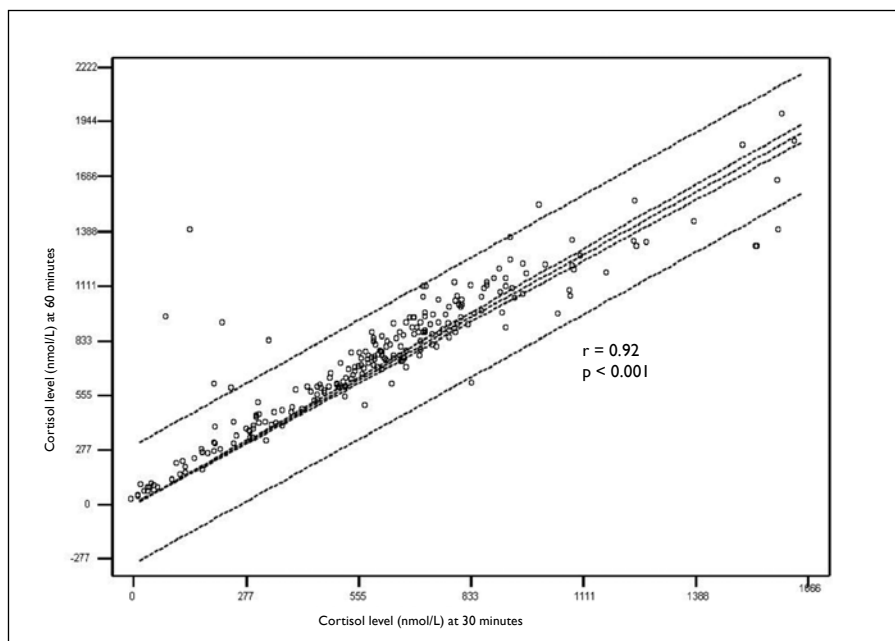
## RESULTS

Out of the 236 study participants, 93 (39%) were males and 143 (61%) were females. The mean age was  $44.4 \pm 21$  years. Plasma cortisol values after administration of 250  $\mu\text{g}$  Synacthen injection are presented in Fig. 1. The cortisol concentration increased significantly from baseline to 30 and 60 minutes after injection of

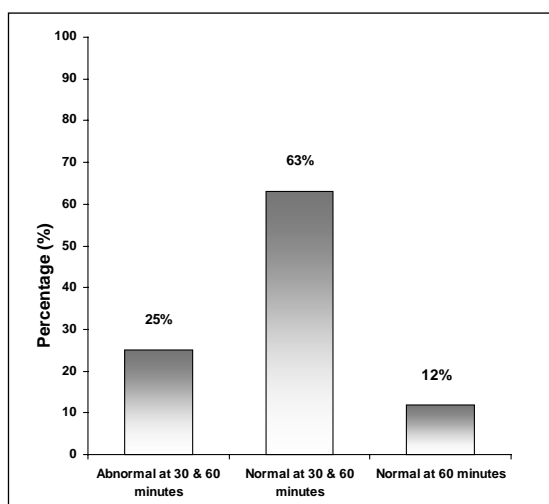


**Fig. 1** Cortisol response 30 and 60 minutes after 250- $\mu\text{g}$  short Synacthen test ( $p < 0.001$  at both 30 and 60 minutes).

Delta 30-basal and delta 60-basal represent the cortisol increment at 30 and 60 minutes after Synacthen injection, respectively.



**Fig. 2** Linear regression and correlation between 30 and 60 minutes post-short Synacthen test.



**Fig. 3** Percentages of normal and abnormal subjects from a total of 236 subjects after the short Synacthen test.

Synacthen ( $p < 0.001$  for each) (Fig. 1). Majority of the subjects reached a cortisol peak, at a value greater than  $20 \mu\text{g/dL}$  ( $555 \text{ nmol/L}$ ) at 60 minutes. There was linear regression, and a strong positive correlation was found between 30 and 60 minutes cortisol values after the Synacthen injection ( $r = 0.92$ ,  $p < 0.001$ ) (Fig. 2). The mean cortisol levels at baseline were  $12.2 \pm 9.9 \mu\text{g/dL}$  ( $339 \pm 275 \text{ nmol/L}$ ), at 30 minutes they were  $22.4 \pm 12.0 \mu\text{g/dL}$  ( $622.2 \pm 333 \text{ nmol/L}$ ), and at 60 minutes they were  $26.6 \pm 13 \mu\text{g/dL}$  ( $738.8 \pm 361 \text{ nmol/L}$ ). There was no significant difference in the increase of cortisol following intravenous or intramuscular injections.

Out of 236 subjects, it was found that SST showed a

normal response in 148 subjects (63.1%) at both 30 and 60 minutes, while 27 subjects (12%) peaked at greater than  $20 \mu\text{g/dL}$  ( $555 \text{ nmol/L}$ ) only at 60 minutes, as the value was less than  $20 \mu\text{g/dL}$  ( $555 \text{ nmol/L}$ ) at 30 minutes. In deficient cases, SST showed abnormal response in 60 cases (25%) at both 30- and 60-minute values (Fig. 3). However, there was only one patient who reached a peak value of  $21 \mu\text{g/dL}$  ( $589 \text{ nmol/L}$ ) at 30 minutes, which decreased to  $17 \mu\text{g/dL}$  ( $485 \text{ nmol/L}$ ) at 60 minutes. To compare with controls, we reported the mean values of these 175 normal subjects in Table I.

## DISCUSSION

AI, although uncommon, is an important clinical problem.<sup>(7)</sup> This problem may result from dysfunction at one or more sites in the hypothalamic-pituitary-adrenal (HPA) axis.<sup>(8)</sup> There is much controversy about which is the most appropriate test for assessing the integrity of the HPA axis.<sup>(19)</sup> Traditionally, the ITT was considered as the gold standard for assessing the integrity of the entire HPA axis as well as the metyrapone test.<sup>(8)</sup> However, both these tests are often inconvenient for the patients and are potentially dangerous as they may precipitate risk of acute AI and hypoglycaemia.<sup>(20,21)</sup> They also have certain limitations; for instance, ITT is contraindicated in elderly patients with cardiovascular diseases, and also in psychiatric or epileptic patients. In some instances, tests may have to be repeated with increased doses to overcome insulin resistance.<sup>(8)</sup>

When using baseline cortisol measurements, diagnosis of AI is often missed because the normal 8 am serum cortisol level includes a broad range, i.e. 5–25

**Table I. Studies reporting cortisol response after the short Synacthen test in controls.<sup>(2)</sup>**

Study	Sex (male/female)	Age (years)	No. of subjects	Cortisol ( $\mu\text{g/ml}$ ) [mean $\pm$ SD or mean (range)]			Method*
				Basal	30 min	60 min	
Wood et al (1965)	25/41	15–45 > 45	32 34	14.4 $\pm$ 4.2 14.9 $\pm$ 3.8	31.7 $\pm$ 5.2 31.0 $\pm$ 5.2		Acid-flourescent steroids
Maynard et al <sup>(32)</sup> (1966)			21	19.2 (6.3–35)		43.9 (28.1–60.3)	Acid-flourescent steroids
Greig et al <sup>(29)</sup> (1966)	9/21	20–73	30+	17.4 $\pm$ 9.2	33.7 $\pm$ 14.0		
Dluhy et al <sup>(31)</sup> (1974)	8/4		12	9.0 (3.0–13.0)	24.0 (15–53)		Radioimmunoassay (RIA)
Willig and Blunck <sup>(18)</sup> (1976)			31	17.2 $\pm$ 1.2	50.5	65.2 $\pm$ 3.3	Competitive binding assay
Grunwald et al <sup>(17)</sup> (1990)	0/42	21–40	42	12.5 $\pm$ 3.4		18.1 $\pm$ 3.9	RIA
Longui et al <sup>(2)</sup> (1998)	64/0	18–45	64	8.8 $\pm$ 3.1	18.7 $\pm$ 2.5	21.2 $\pm$ 2.5	RIA
Present study (2005) <sup>**</sup>	63/112	1–15	17	10.4 $\pm$ 6.2	25.8 $\pm$ 6.37	31.6 $\pm$ 8.67	Flourescence polarisation immunoassay (FPIA)
		16–45	77	14.6 $\pm$ 15	27.1 $\pm$ 13.5	31.7 $\pm$ 12.9	
		> 45	81	20 $\pm$ 17.5	33.7 $\pm$ 27.4	42 $\pm$ 40	

\*Method used for cortisol quantification.

\*\*Note: 175 out of 236 subjects were non-diseased individuals (controls) at 60 minutes.

$\mu\text{g/dL}$  (139–695  $\text{nmol/L}$ ), which is also affected by stress, exercise and food intake.<sup>(7)</sup> Basal plasma cortisol is variable due to its circadian and pulsatile secretion, unless the levels are too high or too low, which obviates the need for further testing.<sup>(2)</sup> Hence, it is an unreliable index of HPA axis normality.<sup>(2)</sup> Similarly, the 24-hour urinary free cortisol excretion test involves the inconvenience of urine collection and an inherently high measurement error at low concentration, precluding its use in clinical evaluation of glucocorticoid deficiency.<sup>(22)</sup> As compared to all these tests, the SST has proven to be quite valuable for detecting clinically-important glucocorticoid deficiency.<sup>(2)</sup> Over the last 30 years, this simple, safe and rapid test has found its place in diagnosing primary and secondary AI, showing a very high specificity but less sensitivity.<sup>(15,23–26)</sup>

However, there is considerable variation in the choice of cortisol cut-off value used for determining normality. Studies show that 60% that of responders consider a maximum cortisol value of greater than 18  $\mu\text{g/dL}$  (500  $\text{nmol/L}$ ), 19.9  $\mu\text{g/dL}$  (553  $\text{nmol/L}$ ) or 21  $\mu\text{g/dL}$  (583  $\text{nmol/L}$ ), as "passable", while for others, acceptable responses range from 14.4  $\mu\text{g/dL}$  (400  $\text{nmol/L}$ ) to greater than 25  $\mu\text{g/dL}$  (694  $\text{nmol/L}$ ).<sup>(27)</sup> Orth et al recommended a plasma cortisol level of greater than 20  $\mu\text{g/dL}$  (555  $\text{nmol/L}$ ) 30 and 60 minutes after SST.<sup>(28)</sup> Stewart et al also recommended a cortisol level of greater than 19.9  $\mu\text{g/dL}$  (553  $\text{nmol/L}$ ) at 30 and 60 minutes.<sup>(3)</sup> In our study, a plasma cortisol level of greater than 20  $\mu\text{g/dL}$  (555  $\text{nmol/L}$ ) was taken as the cut-off value to differentiate between abnormal/subnormal and normal populations.<sup>(7)</sup>

This study determined plasma cortisol response after SST in 236 study subjects. It was found that there was

an increase in plasma cortisol from baseline to 30 and 60 minutes, and the majority of the subjects reached a cortisol peak greater than 20  $\mu\text{g/dL}$  (555  $\text{nmol/L}$ ) at 60 minutes. The maximum plasma cortisol response after injecting Synacthen was found to be positively correlated to the basal cortisol level. This correlation was attenuated with time, probably because the 60-minute value was closer to the maximal ability of the cortex to secrete cortisol than the 30-minute value.<sup>(2)</sup> In this study, SST on the 236 subjects showed that the peak cortisol values were achieved at 60 minutes in a greater number of subjects, as compared to at both 30 and 60 minutes. These findings of our study are consistent with other studies (Table I). In Willig et al's study on 40 patients after SST, peak values were reached at 60 minutes (65.2  $\pm$  3.2  $\mu\text{g/dL}$  [1811  $\pm$  89  $\text{nmol/L}$ ]); and in this report, 60 minutes were considered to be the right moment for blood sampling when using this test.<sup>(18)</sup> In another study by Longui et al on 64 patients, it was found that in most subjects following SST, a maximum response of plasma cortisol was obtained at 60 minutes.<sup>(2)</sup>

The difference between these other studies and our study was that we included subjects of all ages suspected of AI, and we had abnormal (patients) and normal (controls) subjects. The previous studies describing the cortisol response after SST compared patients with chronic diseases and a relatively small number of controls. Chronic diseases included rheumatoid arthritis,<sup>(29,30)</sup> asthma,<sup>(31)</sup> and AI.<sup>(32)</sup> Moreover, the methods used for cortisol analysis in these studies are not currently in use due to lack of method sensitivity, accuracy and precision. The strength of our study include having a good statistical

number of controls and having used the more advanced FPIA method for cortisol measurements (Table I).

This study showed that measuring the 60-minute cortisol level after Synacthen injection was reliable enough in identifying normal subjects for excluding AI, while being equally effective in identifying abnormal cases, as compared to measurements taken at both 30 minutes and 60 minutes. Therefore, it is suggested that a single 60-minute post-Synacthen serum cortisol level may suffice as a reliable screening test for AI, as compared to the conventional SST. This also signifies its cost-effectiveness, especially in third world countries, where cost is a major issue for diagnosing and treating patients.

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