

Comparative evaluation of 30 and 60 minutes cortisol levels during short synacthen test for diagnosis of adrenal insufficiency

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Abstract

Objective: To assess and compare diagnostic value of 30-minute cortisol level over 60-minute level in the diagnosis of adrenal insufficiency.

Methods: The comparative cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from August 2017 to May 2018, and comprised patients referred to the facility for short synacthen test with suspicion of adrenal insufficiency. Blood samples for serum cortisol were taken at time-0 and then 30 and 60 minutes after the adreno-cortico-tropic hormone injection. Total serum cortisol was measured. Adrenal insufficiency was defined as stimulated cortisol level <500 nmol/l at 30 and 60 minutes post-stimulation. SPSS 24 was used for data analysis.

Results: Of the 111 subjects, 56(50.4%) were males and 55(49.5%) were females. Overall mean age was 34 ± 20 years. Mean basal serum cortisol level was 110 ± 98 nmol/l in patients with adrenal insufficiency and it was 294 ± 164 nmol/l in patients with intact adrenal functions. Cortisol level at both 30 and 60 minutes was significant ($p < 0.001$). Receiver Operating Characteristics curve was plotted which showed area under curve of 0.83 and 0.82 for 60 and 30 minutes respectively.

Conclusion: The 30-minute cortisol level post-stimulation carried no diagnostic value. Measuring cortisol level once at 60-minute post-stimulation would be of more value apart from being cost-effective in the diagnosis of adrenal insufficiency.

Keywords: Adrenal insufficiency, ACTH stimulation test, Post-ACTH stimulation, Cortisol levels. (JPMA 69: 1628; 2019). doi: 10.5455/JPMA.299484.

Introduction

Adrenal insufficiency is a rare and potentially fatal condition.¹ It has a reported prevalence of 0.01% with about 40 to 110 cases of primary adrenal insufficiency per million in the United States (US), and 93 to 140 cases per million in the European region. The prevalence of secondary adrenal insufficiency has been reported to be 150 to 280 cases per million in Europe, and there has been an increase in incidence in the past few decades.² It occurs either due to decreased production or defective function of glucocorticoids and may be accompanied by decreased production of aldosterone and adrenal androgens.³ Clinical picture may be due to primary adrenal insufficiency or as a consequence of defective hypothalamic-pituitary-adrenal axis (i.e., secondary adrenal disease). Patients suffering from adrenal insufficiency present with very non-specific symptoms, such as weakness, lethargy,

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pigmentation, weight-loss, hypotension, hypoglycaemia and abdominal discomfort, making its correct diagnosis a big challenge which is often delayed.⁴ Prompt diagnosis is critical in the management of patients because, if missed, adrenal insufficiency can be life-threatening with significant morbidity and mortality. Insulin tolerance test (ITT) is considered the gold standard for diagnosis of adrenal insufficiency. As it is expensive, requires close monitoring and is not safe, especially in elderly patients and individuals with history of seizures and cardiac disorders, Short Synacthen Test (SST) is offered as a suitable alternative for diagnosing adrenal insufficiency. SST protocol includes withdrawing of blood samples for cortisol at time-0, 30 and 60 minutes post-stimulation with $250\mu\text{g}$ synthetic intramuscular (IM) adreno-cortico-tropic hormone (ACTH) injection. However, there is still controversy whether diagnosis of adrenal insufficiency by SST may be facilitated by considering single blood sample for cortisol post-ACTH injection instead of two

blood samples. Since single cortisol value would be more convenient to the patient and also cost-effective, research has been done in different populations to assess the utility of 30-minute cortisol value for provision of any incremental diagnostic value to a single value of cortisol level after 60 minutes. Local data is sparse in this regard. The current study was planned to compare 30- and 60-minute cortisol levels during SST, and to assess if the 30-minute value was of any additional help in adrenal insufficiency diagnosis.

Patients and Methods

The comparative cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from August 2017 to May 2018. After approval from the institutional review board, the sample size was calculated using World Health Organisation (WHO) calculator,⁵ taking prevalence of 0.01%² and margin of error 2%. Patients who were referred to AFIP for assessment of unexplained symptoms, like postural dizziness, hypotension, fatigue, weight-loss or pigmentation, were included using non-probability convenience sampling. Patients who were on steroid treatment or oral contraceptive pills, having had pituitary surgery in the preceding three months and pregnant women were excluded. Samples were taken only after taking informed consent from the subjects.

Baseline 2.0ml blood sample was taken for serum cortisol at 0900h. Then 250µg of synthetic ACTH was administered IM to patients >2 years of age. In case of children <2 years of age, 15µg/kg body-weight of synthetic ACTH was administered. Later, 2.0ml blood samples were taken each at 30 and 60 minutes post-ACTH. Serum was separated and stored at -20°C till assay of cortisol. Total serum cortisol was measured on competitive immunoassay using direct chemiluminescence technique (ADVIA Centaur® XP, Random Access Chemiluminescence Immunoassay System, Siemens Health Care Diagnostics Inc). Data was analysed using SPSS 24. Kolmogorov-Smirnov test was applied to check normality of data. Descriptive statistics, mean±standard deviation (SD) and frequencies and percentages were computed. Median and interquartile range (IQR) were calculated for post-stimulation cortisol levels. T test was used to analyse basal cortisol level whereas 30- and 60-minute levels were analysed using Mann-Whitney Test. $P \leq 0.05$ was considered statistically

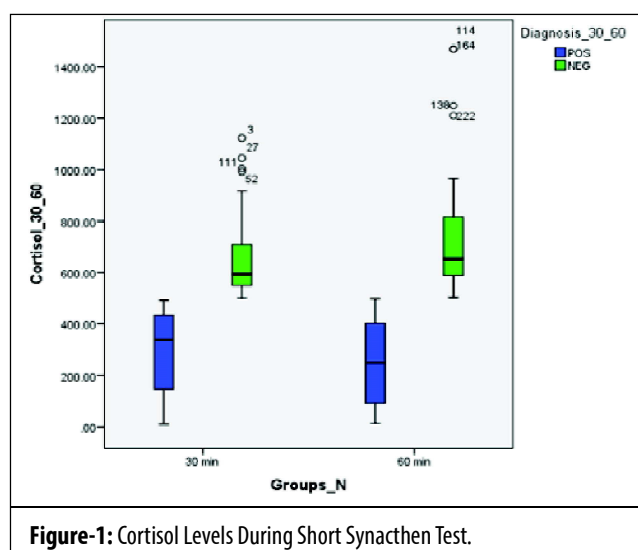


Figure-1: Cortisol Levels During Short Synacthen Test.

Table: Diagnostic accuracy of 60 and 30 minutes cortisol levels.

Parameter	60 minutes cortisol level as gold standard	30 minutes cortisol level as gold standard
Sensitivity (%)	95.35	68.33
Specificity (%)	72.06	96.08
Positive Likelihood ratio	3.41	17.43
Negative Likelihood ratio	0.06	0.33
Positive Predictive value	68.33	95.35
Negative Predictive Value	96.08	72.06

significant. Adrenal insufficiency was defined as stimulated cortisol level <500 nmol/l at 30 and 60 minutes post-ACTH.

Results

Of the 111 subjects, 56(50.4%) were males and 55(49.5%) were females. Overall mean age was 34 ± 20 years. Of the total, 78(70.3%) subjects had normal basal cortisol levels, while 33(29.7%) had adrenal insufficiency. Normal response at both 30 and 60 minutes post-ACTH was seen in 48(43.2%) subjects, while 42(37.8%) showed abnormal response at both time slots. There were 20(18%) subjects with cortisol level >500 nmol/l only at 60 minutes with an inadequate response at 30 minutes post-stimulation. There was only 1(0.9%) patient who showed adequate response at 30 minutes and inadequate response at 60 minutes. Box plot analysis of basal and post-ACTH cortisol levels was done (Figure 1).

Mean basal serum cortisol level was 110 ± 98 nmol/l in patients with adrenal insufficiency and it was 294 ± 164 nmol/l in patients with intact adrenal functions. Basal

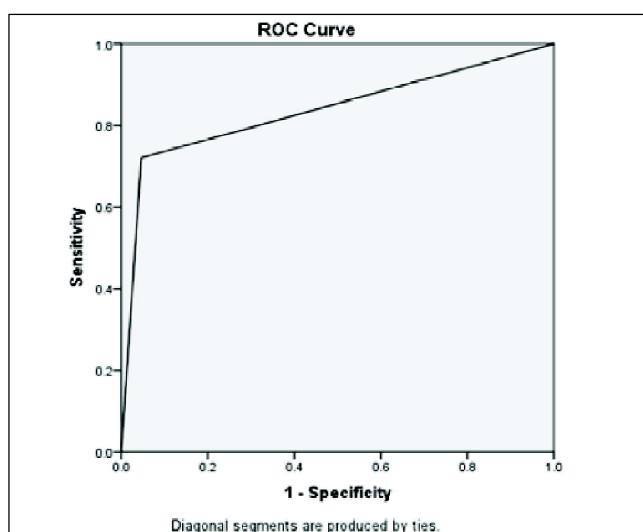


Figure-2: Receiver Operating Characteristics (ROC) curve with 60-minute cortisol level as gold standard

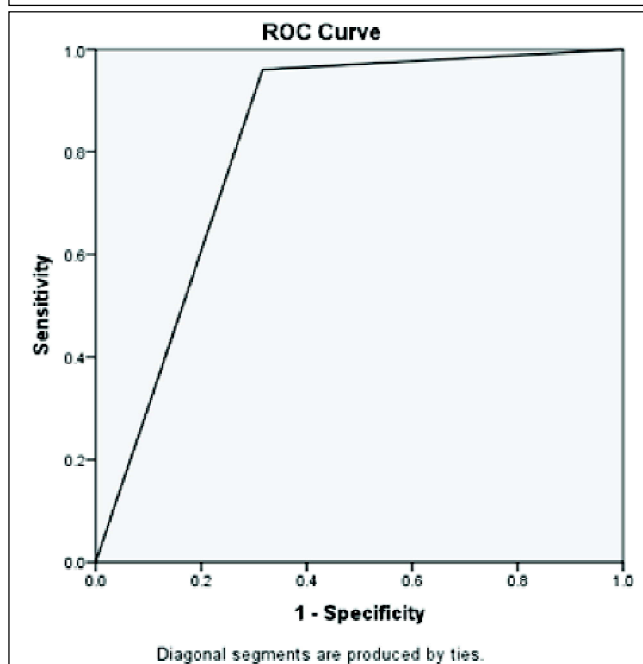


Figure-3: Receiver Operating Characteristics (ROC) curve with 30-minute cortisol level as gold standard.

cortisol level was significant ($p < 0.001$), and so were the values at 30 and 60 minutes ($p < 0.001$). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 30-minute cortisol level were done while keeping the 60-minute value as the gold standard (Table). Receiver Operating Characteristics (ROC) curves were plotted for both 30-minute and 60-minute cortisol values (Figures 2-3).

Discussion

Adrenal insufficiency is recognised as a serious disorder with its diagnosis still challenging and which remains under debate.^{6,7} SST is an essential investigation done during patients' workup with suspicion of adrenal insufficiency.^{8,9} The current study was conducted to compare and evaluate both 30- and 60-minute cortisol levels post-ACTH stimulation and to compare the peak value of cortisol at 30 and 60 minutes in the diagnosis of adrenal insufficiency. Mean age of patients with adrenal dysfunction was 34 ± 20 years. It was comparable with the reported mean age of 34.78 ± 11.85 year.¹⁰ The current study found that about 18% subjects would have been falsely diagnosed, if only 30-minute cortisol level was considered, and 0.3% patients would have been incorrectly labelled with adrenal insufficiency if only the 60-minute cortisol value post-ACTH would have been considered. These results are in concordance with findings of a study carried out in Canada¹¹ in which false positive rate with 30-minute level was 13% and with 60-minute it was 0.3% which is what the current study found. Response of SST seen in the current study is similar to a study conducted in Caucasian population¹² which recommended that if only 30-minute cortisol level is considered, it would result in improper diagnosis. Recommendations suggested by these studies are supported by the fact that it takes about 60 minutes to reach peak cortisol level post-ACTH. After that usually a plateau is attained.¹³ Thus, an incorrect diagnosis would eventually lead to undue use of glucocorticoids, leading to serious effects, including osteoporosis, diabetes mellitus and cardiovascular complications.^{14,15} Median cortisol levels at 30 and 60 minutes of the current study were in concordance with an earlier study.¹⁰ Higher value at 60 minutes signifies the fact that more patients would be having cortisol level above the cut-off value of 500 nmol/l than at 30 minutes. Thus, considering 60-minute cortisol level would in turn lead to a more accurate diagnosis compared to a single measurement at 30 minutes.

Mean basal cortisol level (110 ± 98 nmol/l) of patients who were eventually diagnosed with adrenal insufficiency were lower compared to that (294 ± 164 nmol/l) of patients in whom adrenal insufficiency was ruled out after SST. These findings showed that lower basal cortisol level was predictive of adrenal insufficiency. These results are in line with earlier findings¹⁶ which showed a diagnostic accuracy of basal cortisol level in patients suffering from

adreno-cortical defect. A study¹⁷ reported that cortisol level of 60 minutes post-ACTH was comparable to 24-hour cortisol sampling. Majority of subjects reached maximum cortisol level at 60 minutes which is also illustrated in the present study. A study carried out in King's College Hospital, London,¹³ reported sensitivity of 91% and area under curve (AUC) of 0.96 while keeping 60-minute cortisol level as the gold standard which are comparable to the sensitivity (95%) and AUC (0.83) in the present study. ADVIA Centaur® XP, Random Access Immunoassay System was used in both studies to measure serum total cortisol level.

The fact that the present study had a good sample size with a low margin of error and a good representation of diseased individuals along with a sensitive, precise and accurate method of analysis of serum cortisol level by chemiluminescence immune assay adds up to the strengths of the current study.

The limitations of the current study are that SST results were not compared with that of the suggested gold standard i.e., ITT and the subjects included were patients with suspicion of adrenal dysfunction while primary and secondary adrenal insufficiency were not separately addressed.

Conclusion

The 30-minute cortisol level post-ACTH in SST did not offer any additional diagnostic information. Consideration of only 30-minute level instead of 60-minute cortisol level would lead to false positive diagnosis, resulting in undue treatment. Measuring cortisol level at 60 minutes only during SST would be more convenient, reliable and cost-effective in the diagnosis of adrenal insufficiency.

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Conflict of interest: None.

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