Original Article

Predicting the result of 250 μg ACTH stimulation test by the morning cortisol level

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Abstract **Predicting the result of 250 μg ACTH stimulation test by the morning cortisol level**

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Background and Aims: Morning cortisol level is the test for diagnosis of adrenal insufficiency(AI). When the result of morning cortisol level was inconclusive, the 250 μg ACTH stimulation test was used. However the correlation between the morning cortisol and the cortisol response in the ACTH stimulation test has not been studied. So we studied to find out the morning cortisol level that could predict the result of ACTH stimulation test.

Material and methods : This study was retrospective descriptive study. All adult patients who were performed ACTH stimulation test in Prapokklao hospital from June 2006 through october 2008 were recruited. The correlation between the morning cortisol and the results of the ACTH stimulation test were determined. The ROC curve was used to find out the best morning cortisol level for the highest sensitivity and specificity of the ACTH stimulation test.

Results : One hundred and seventy-seven ACTH stimulation tests were done during that time. There were 135 morning cortisol levels which were inconclusive (3-18 mg/dl). The morning cortisol levels

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were highly correlated to the peak cortisol responses after the ACTH stimulation test. The morning cortisol levels which were below 8.25mg/dl can predict the negative result of the response after the ACTH stimulation test (peak cortisol level ? 18 mg/dl) with 78.8% of sensitivity , 80% of specificity, 73.24% of positive predictive value and 84.62% of negative predictive value (AUC=0.854). The factors which correlated to the cortisol levels after ACTH stimulation test were the history of steroid usage (P<0.001), cushingoid appearance (P<0.001) and eosinophilia (P=0.002).

Conclusions: The morning cortisol level which was below 8.25mg/dl was the predictive negative result of the response after the ACTH stimulation test, especially in patient who had the history of chronic steroid usage, cushingoid appearance and eosinophilia.

Key word : ACTH Stimulation test, Adrenal insufficiency, morning cortisol level,Diagnosis

INTRODUCTION

Adrenal insufficiency is characterized by impaired cortisol secretion as a result of decrease of hypothalamus, pituitary or adrenal gland itself. The clinical features are related to the rate of onset, cause and severity of adrenal insufficiency. The patient may present with vague features e.g. tiredness, nausea, vomiting, arthralgia and weight loss. Clinical suspicion of the diagnosis should be confirmed with definitive diagnostic tests. The measurement of basal plasma cortisol can be used to exclude the diagnosis if it is greater than 18 μg/dl. Adrenal insufficiency was diagnosed if the basal plama cortisol is lower than 3 μg/dl. In practice, the basal or morning cortisol are foten in the inconclusive range that required the dynamic function test of HPA axis .

The reference test for establishing the integrity of HPA axis require assessing the response to either a strong stimulus (e.g. Insulin-induced hypoglycemia) or an interruption of the negative feedback from cortisol (overnight metyrapone test). However, these reference tests have major drawbacks.[1]

Insulin-induced hypoglycemia (IIH) test is the major gold standard for diagnosis the adrenal insufficiency, but it is the unpleasant test due to many contraindications and serious side effect. It is contraindicated in the elderly and those with a history of seizure or cardiovascular disease and requires adrenergic or neurological symptoms.[2]

The overnight metyrapone test was

introduced by Liddle et al in 1959. When IIH test is contraindicated and where the compound is available, the short metyrapone test (given as 30 mg/kg orally at midnight with measurement of cortisol and 11-deoxycortisol concentration at 8.00 am. Of the follow morning) may be perform.[3] This test carries a risk of adrenal crisis, errors can occur from other drugs affecting clearance of metyrapone[4] and require special lab for measure 11-deoxycortisol concentration. Moreover, metyrapone is not available in Thailand.

ACTH stimulation test is alternative test that are quicker, cheaper, safer and more available in general hospital. The rational for using the corticotropin analog stimulation test is the assumption that in chronic endogenous corticotropin deficiency, acute responsiveness of the adrenal zona fasciculata is diminished and fails to mount an adequate cortisol response.[5] But the result of ACTH stimulation test depend on degree and duration of ACTH deficiency which determine the degree of adrenal atrophy.[3]

Standard ACTH stimulation test is performed by administering intravenous 250 μg of cosyntropin. The cortisol level was measured before cosyntropin injection, 30 min and 60 min after cosyntropin injection.[6,7] Various cut off levels or criteria were proposed.[3, 6, 8] However the correlation between the basal cortisol levels including the clinical and the cortisol response in the ACTH stimulation test has not been studied. **Objective :** To evaluate the relationship between the basal or morning cortisol levels and the cortisol reponses after the ACTH stimulation test in evaluation of adrenal insufficiency.

Materials and Methods

Study design

The retrospective study was done in Prapokklao Hospital which are the tertiary medical center at Chanthaburi province, Thailand.

Population characteristics

All adult patients who were performed 250 microgram ACTH stimulation test during June 2006 through october 2008 were included in this study.

Data collection

The data which are sex, age, primary diagnosis, co-morbidities, drugs (which can affect the cortisol level measurement), history of steroid usage, cushingoid appearance, status (inpatient or outpatient), basic blood chemistry, cause of investigation, cortisol level and the results of ACTH stimulation test were review from OPD cards and IPD charts.

Reference test

The diagnosis of Hypothalamus-Pituitary-Adrenal axis insufficiency (HPAI) was based on an abnormal cortisol response to

standard dose ACTH stimulation test (peak stimulated cortical level < 18 mcg/dL)[6, 9]

Cortisol assay

The cortisol assay methods used in Prapoklao hospital is electrochemiluminescence immunoassay (ECLIA) method. (COBAS®, Roche diagnostics)

Standard dose ACTH stimulation test

The synthetic corticotropin analogues, Tetracosactide hexaacetate (Cortrosyn®, Organon Oss Holland.) was administered intravenously at a dose of 250 mcg and serum cortisol levels were obtained at baseline, 30 minutes and 60 minutes after injection.[6]

Basal cortisol

The basal or morning plasma cortisol level was defined by the plama cortisol that performed in the morning at 8.00 am.

Statistical analysis

The data were analyzed by SPSS version 14.0

The Receiver-Operator-Characteristic (ROC) curve analysis was created to evaluate the performance of basal cortisol in predicting the results of the 250 μg ACTH stimulation test (standard reference test).

Positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity and accuracy were calculated.

PPV (calculated as TP/(TP+FP)) was defined as likelihood that a subject with a positive test was adrenal insufficiency (an abnormal response to ACTH stimulation test).

NPV (calculated as TN/(TN+FN)) was defined as the likelihood that a subject with a negative test (cortisol > cut off) was not adrenal insufficiency (a normal response to ACTH stimulation test).

Sensitivity (TP/(TP+FN)) and specificity (TN/(TN+FP)) were defined as percentages of patients who had peak cortisol value below and above the specific cut off point, respectively.

Accuracy ((TP+TN)/(TP+FP+TN+FN)) was defined as the percentage of patients that were correctly identifies as adrenal insufficiency of no adrenal insufficiency.

The factors that may correlate to the result of standard dose ACTH stimulation test was analyzed by independent sample T test (data which is normal distribution) or non-parametric (2-independent sample) test (data which is not normal distribution). A P value of < 0.05 was considered significant.

Result

Patient characteristic

177 patients were performed ACTH stimulation test between June 2006 through october 2008. Thirty six point seven percents of the patients were diagnosed adrenal insufficiency by abnormal cortisol response after ACTH stimulation test. The most of patients were diagnosed of secondary

adrenal insufficiency (72.48%). The details of diagnosis was shown in table 1 and graph 1. Most patients were performed the test in in-patient setting. The mean age was 63.52±15.39 year. Sixty eight point four percents of patients of patients were female. Most patients had co-morbidities (84.2%). The most common co-morbidities were hypertension (30.5%) and DM (28.8%). The details of co-morbidities were shown in graph 2. The patients who had history of steroid usage was found in 39.4%. The most common of steroid which was used were herbal medicine (53.5%). The cushingoid appearances were documented in only 19.2%. The details of other patient characteristics and the laboratories were shown in table 2.

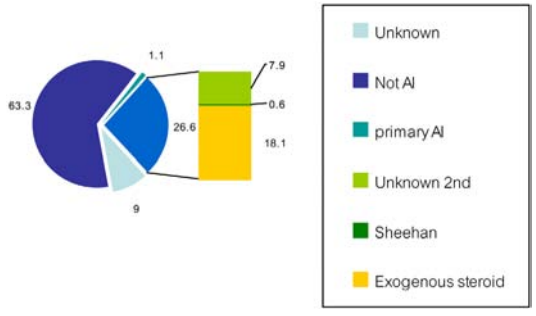
Table 1 : result and diagnosis of study population (n=177)

Result of ACTH stimulation test	Percent
● Normal cortisol response	63.3
● Abnormal cortisol response	36.7
Diagnosis of adrenal insufficiency(AI)*	**100**
● Primary AI	3
● Secondary AI	72.48
○ SAI due to exogenous steroid	49.32
○ Sheehan's syndrome	1.63
○ SAI unknown cause**	21.53
● Unclassified AI**	24.52

* *Diagnosis based on result of ACTH stimulation test & ACTH level.*

** *Due to loss to follow up, death & incomplete diagnostic work up.*

Graph 1: Final diagnosis of the patients performed ACTH stimulation test in PPK hospital



Graph 2: comorbidity of the patients performed ACTH stimulation test

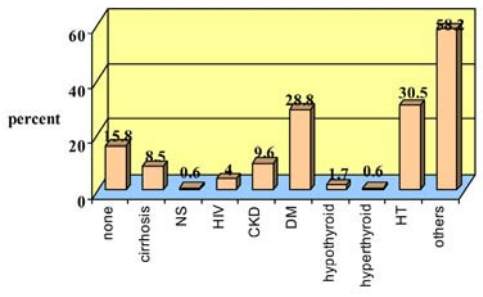


Table 2 : Patient characteristics of study population in Prapokklao hospital.

Patient characteristics	Result			
	overall	Response to ACTH stimulation test		
		Normal	Abnormal	P value
• age	63.52±15.39	61.88	66.27	0.05
• sex				0.169
a. female	68.4%	66.1%	33.9%	
b. male	31.6%	55.4%	44.6%	
• Medication				
a. No medication use	88.1%			
b. Medication use	11.9%			
i. Amiodarone	0.6%			
ii. NDHP-CCB	0.6%			
iii. Azole	1.1%			
iv. Macrolides	2.8%			
v. Phenytoin	1.1%			
vi. Nevirapine	1.1%			
vii. Rifampicin	5.1%			
• History of steroid usage	**39.4%**	**40.6%**	**59.4%**	**<0.01**
a. Therapeutic	10.2%			
b. Herbal medicine	53.5%			
c. Self-medication	27.7%			
d. Unclassified	8.6%			
• Cushingoid apearance	**19.2%**	**26.5%**	**73.5%**	**<0.01**
• Patient status				0.428
a. Inpatient	70.5%	61.0%	39.0%	
b. Outpatient	30.5%	67.3%	32.7%	

• CBC				
a. Hct	32.26	32.47	31.93	0.590
b. WBC	8983.58	8981.85	8986.25	0.996
i. neutrophil No.	6065.20	6205.43	5848.30	0.626
ii. lymphocyte No.	1697.48	1698.19	1696.23	0.990
iii. eosinophil No.	401.44	297.19	562.70	0.006
c. eosinophilia (% of patient)	**23.2%**	**14.4%**	**37.9%**	**0.001**
• BUN	17.57±15.67	16.92	18.54	0.517
• Cr	1.30±0.93	1.21	1.44	0.127
• Sodium	134.55±7.46	133.70	135.60	0.464
• Potassium	3.72 ±0.80	3.75	3.67	0.514
• Chloride	101.49±9.43	101.29	101.82	0.731
• Bicarbonate	23.85±4.46	23.66	24.16	0.495
• Hyponatremia : Na<135 mEq/dl (% of patient)	**42.4%**	**34.6%**	**55.3%**	**0.026**
• Corrected Calcium	9.62 ±1.07	9.57	9.72	0.587
• Blood sugar	116.37±54.55	117.44	114.57	0.772
• Albumin	**2.89 ± 0.75**	**3.11**	**2.58**	**0.001**
• Protein	6.59 ±1.09	6.79	6.34	0.104

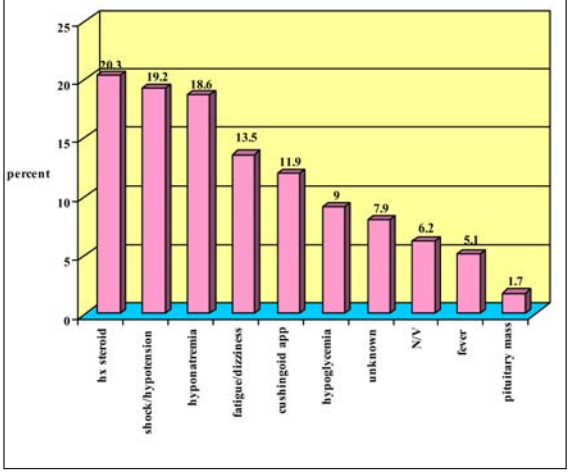
Primary diagnosis

Majority of the patients were not diagnosed adrenal insufficiency (63.3%). Among the patients who were diagnosed adrenal insufficiency, secondary adrenal insufficiency was the most common (72.48%). The exogenous steroid usage was the most common cause. Primary adrenal insufficiency was found in 1.1% (2 patients) which were acute bilateral adrenal hemorrhage due to DIC (1 patient) and disseminated fungal infection in AIDS patient (1 patient)

Causes of investigation

The reasons of decision to perform ACTH stimulation test are shown in graph 3. Three most common causes of investigation were history of steroid usage (20.3%), unknown of shock or hypotension (19.2%) and hyponatremia (18.6%)

Graph 3 : Reasons of decision to perform ACTH stimulation



Cortisol level

The cortisol level before performing ACTH stimulation test was classified into 2 groups; basal morning cortisol level (8.00 am) and cortisol level during shock (blood pressure <90/60). The mean of basal cortisol level were 9.11±3.81 mcg/dl and the mean of corisol level during shock were 16.14±12.78.

The mean of peak cortisol level was 22.22±11.59 mcg/dl and the absolute cortisol increment was 11.56±7.92 mcg/dl after ACTH stimulation test. The details were shown in table 3.

Table 3: cortisol level before ACTH stimulation test & after ACTH stimulation test.

	Cortisol level (mcg/dl±SD)
Cortisol level before ACTH stimulation test	9.80±5.69
● 8.00 am cortisol level	9.11±3.81
● Cortisol level at shock or hypotension	16.14±12.78
ACTH stimulation test	
● Cortisol level	
○ 8.00 am cortisol level	10.80±6.95
○ 8.30 am cortisol level	18.72±8.56
○ 9.00 am cortisol level	22.10±11.60
○ Peak cortisol level	22.22±11.59
○ Absolute cortisol increment	11.56±7.92

There were 37.3% and 37.9% of the patients was diagnosed of adrenal insufficiency when we used the criteria for diagnosis of adrenal insufficiency in patient who had peak serum cortisol level after ACTH stimulation test <18 mcg/dl or the absolute cortisol increment <9 mcg/dl after ACTH stimulation test respectively. The mean of basal cortisol level in patients who had diagnosed adrenal insufficiency by peak serum cortisol level after ACTH stimulation test was 5.67±3.41 mcg/dl and lower than patients who did not have diagnosed adrenal insufficiency which was 13.84±6.73 mcg/dl significantly with p value <0.001. The mean of basal cortisol level in patients who had diagnosed adrenal insufficiency by absolute cortisol increment <9 mcg/dl after ACTH stimulation test was 9.15±7.73 mcg/dl and lower than patients who did not have diagnosed adrenal insufficiency which was 11.86±6.21 mcg/dl significantly with p value 0.012. The mean of basal cortisol level in patient who had adrenal insufficiency by 2 criteria were shown in table 4.

Table 4: The mean of basal cortisol level comparison between patient diagnosed HPAI from ACTH stimulation test with 2 diagnostic criteria.

Diagnostic criteria: defined by peak stimulated cortisol level < 18 mcg/dl		
group	**8.00am cortisol level**	**P value**
● peak stimulated cortisol level ≥ 18	13.84±6.73	<0.001
● peak stimulated cortisol level < 18	5.67±3.41	
Diagnostic criteria: defined by absolute cortisol increment < 9 mcg/dl		
group	**8.00am cortisol level**	**P value**
● absolute cortisol increment ≥ 9	11.86±6.21	0.012
● absolute cortisol increment < 9	9.15±7.73	

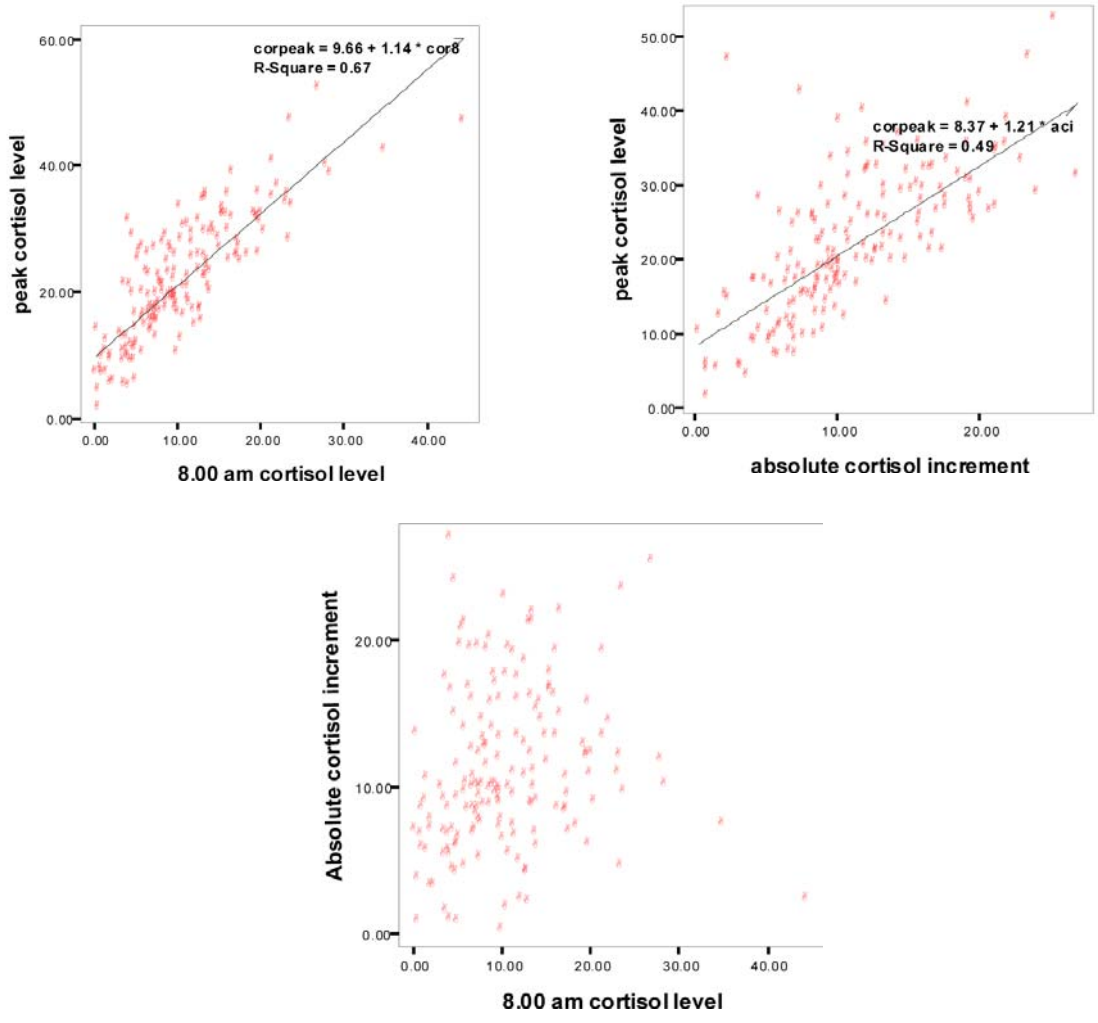
The factors which correlate to the peak of cortisol levels after ACTH stimulation test were the history of steroid usage (p value <0.001), the presence of cushingoid appearance (p value <0.001), the presence of eosinophilia (absolute eosinophil ≥500 cells/cu.mm.)(p value 0.002), the ratio of BUN and Cr (BUN/Cr ≥20) (p value 0.044), and serum albumin(p value 0.006),. The correlation between any factors and the peak of cortisol response were shown in table 5.

Table 5: factors that influenced to peak cortisol level after ACTH stimulation test.

Factors	8.00 am cortisol level	P value	Peak cortisol level	P value
Sex		0.268		0.991
• Male	10.36±6.30		22.21±9.38	
• Female	11.76±8.17		22.23±15.42	
Hx of steroid use		<0.001		<0.001
• Yes	8.19±6.61		17.60±8.77	
• No	12.47±6.67		25.31±12.20	
Cushingoid appearance		<0.001		<0.001
• Yes	6.42±5.21		15.27±7.40	
• No	11.81±6.93		23.96±11.84	
Leukocytosis		0.027		0.365
• ≥10,000	12.97±9.22		23.30±11.29	
• <10,000	9.78±5.63		21.50±12.07	
Eosinophilia		0.003		0.002
• ≥500	7.97±5.76		17.09±8.53	
• <500	11.73±7.26		23.74±12.32	
Serum sodium		0.660		0.095
• ≥135	9.37±3.70		21.89±7.24	
• <135	9.69±4.29		19.62±7.71	
Corrected Calcium		0.624		0.456
• ≥10.5	13.04±10.01		20.67±9.74	
• <10.5	11.72±7.16		23.24±9.60	
BUN/Cr ratio		0.001		0.044
• ≥20	12.19±3.54		19.57±6.78	
• <20	8.73±4.02		23.30±8.00	
Serum albumin		0.713		0.006
• < 3.5	9.45±3.64		19.20±7.20	
• ≥3.5	9.81±4.09		24.37±6.15	

The basal cortisol level was highly correlate to the peak cortisol level (r=0.67). The absolute of cortisol increment was correlate to the peak of cortisol level (r=0.49) but it was not correlate to the basal cortisol. The correlation between basal cortisol levels, the peak cortisol level and the absolute of cortisol increment were shown in graph 4.

Graph 4: correlation between 8.00 am cortisol level, peak cortisol level & absolute cortisol increment of the study population.



The ROC curve was done to find the optimal cortisol level that should be use for predicting the result of ACTH stimulation test and was shown in graph 5. The cut-point cortisol level was 8.25 mcg/dl with 78.8% sensitivity, 80% specificity, 73.42% positive predictive value, 84.62% negative predictive value and 79.19% accuracy of the result of ACTH stimulation test as shown in table 6.

Graph 5: Reciever operating characteristic curve (ROC Curve)

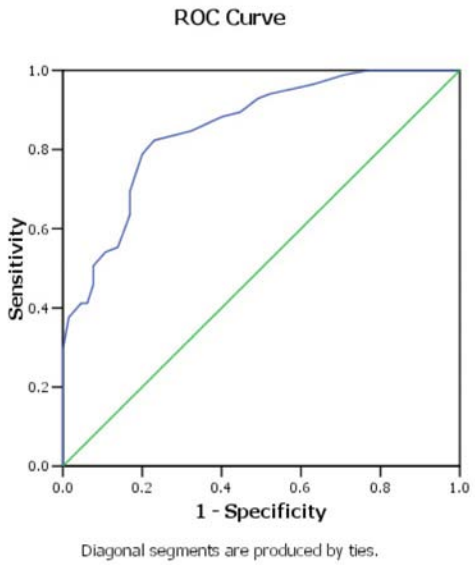


Table 6: The optimal cortisol level and the predictive value to diagnose adrenal insufficiency

Parameters	Value
Area Under the Curve (AUC)	0.854
The Cutoff point of basal cortisol level	8.25 mg/dl
• Sensitivity	78.8%
• Specificity	80%
• Positive predictive value	73.42%
• Negative predictive value	84.62%
• Accuracy	79.19%

The positive predictive value was improve when we combine the basal cortisol levels with the factors that significantly affected the peak cortisol level after ACTH stimulation test. The PPV was increase from 73.42% to 78.8%, 82.6%, 84.2% and 85.7% when combine with history of steroid usage, Hyponatremia (Na<135 mEq/dl), eosinophilia and the presence of cushingoid appearance respectively as shown in table 7.

The possibility of the normal cortisol response after the ACTH stimulation test is 100% if the basal cortisol level is more than 13.20 mcg/dl. And nobody had the normal cortisol response after the ACTH stimulation test if the basal cortisol level was below 3.54 mcg/dl, 4.06 mcg/dl with the presence of cushinoid appearance, 4.09 mcg/dl with eosinophilia and 4.18 mcg/dl with history of steroid usage.

Table 7: The combination between basal cortisol level and other factors in predicting the result of ACTH stimulation test.

Factors that used for predict the response	Percent of patients with abnormal response ACTH stimulation test
Basal cortisol level < 8.25 mg%	73.42%
Basal cortisol level < 8.25 mg% and The history of steroid usage	78.8 %
Basal cortisol level < 8.25 mg% and Hyponatremia (Na<135 mEq/dl)	82.6 %
Basal cortisol level < 8.25 mg% and Eosinophilia	84.2 %
Basal cortisol level < 8.25 mg% and presence of cushingoid appearance	85.7 %

DISCUSSION

The measurement of basal plasma cortisol is simple and useful for diagnosis of adrenal insufficiency when the level is lower than 3 μg/dl. The diagnosis of adrenal insufficiency should be exclude when the basal cortisol level is greater than 18 μg/dl. But in many circumstances the basal morning cortisol are in the inconclusive range that required the dynamic function test of HPA axis.

The insulin induced hypoglycemia test (IIH) was the gold standard dynamic test for diagnosis of adrenal insufficiency which Plumpton and Besser showed the normal cortisol response after ITT since 1969.[10] But the IIH has several limitations for example it is labor intensive, requires medical supervision and can be hazardous particularly in young children [11] and in adults with seizure disorders or heart disease, and adequate hypoglycemia is not achieved in some cases.[12]

The ACTH stimulation test which is the alternative test was used increasingly. It could assess the integrity of the HPA axis and be simple, cheap, efficient, less side effect and the accuracy is optimal. Lindholm evaluate the clinical value of the 30 minutes ACTH test in assessing the hypothalamic-pituitary-adrenocortical function[13]. They found a significant correlation (R=0.83) between the result of IIH test and the 30-minuteACTH test (except shortly after acute ACTH deprivation). The later review [3, 6] supported the usefulness of ACTH stimulation test in evaluation of hypothalamus-pituitary-adrenal

integrity with promising specificity (95%) and sensitivity (97% and 57% in primary and secondary adrenal insufficiency, respectively). The ACTH stimulation test in secondary adrenal insufficiency relies on assumption that chronic ACTH deficiency result in adrenal atrophy and hence hyporesponsiveness to exogenous administration of synthetic ACTH.[1,5,14] Major concern of ACTH stimulation test is a high rate of false normal responses, especially in patients with mild secondary adrenal insufficiency or recent onset of secondary adrenal insufficiency[15,16,17,18], but some authors found that ACTH stimulation test possesses a high predictive value in excluding ACTH deficiency in patients undergoing Pituitary Surgery and/or radio therapy.[19] While others showed a long-term predictive accuracy and safety with reference to clinical outcome.[20]

Although ACTH stimulation test has many advantages, in real life practice, ACTH stimulation test can be performed only in tertiary hospitals where the Cortrosyn and cortisol assay are available. Primary hospital should have a tool for making a decision to refer suspected patients to tertiary hospital for performing ACTH stimulation test with a simple clinical data, basic laboratory value and the basal cortisol level. Our retrospective study showed that the basal cortisol level which was more than 8.25 mcg/dl had 78.8% sensitivity, 80% specificity, 73.42% positive predictive value, 84.62% negative predictive value and 79.19% accuracy of the result of ACTH stimulation test. When the basal cortisol level was below 3.54 mcg/dl, 4.06 mcg/dl with the presence of cushingoid appearance, 4.09 mcg/dl with eosinophilia and 4.18 mcg/dl with history of steroid usage, the abnormal of ACTH stimulation test can be predictive. On the other way if the basal cortisol level is more than 13.20 mcg/dl, the normal response after ACTH stimulation test can be predictive.

Our study showed that the prevalence of patient characteristics e.g. history of steroid usage, the cushingoid appearance, hyponatremia (Na<135 mEq/dl), eosinophilia were increased significantly in the adrenal insufficiency group. The prevalence of hypercalcemia in adrenal insufficiency group was increased in some studies but not in our study. So we considered the significant factors that could be increase the positive predictive values. The PPV was increase from 73.42% to 78.8%, 84.2% and 85.7% when combine with history of steroid usage, Hyponatremia, eosinophilia and the presence of cushingoid appearance respectively. Even though hypoalbuminemia was correlate to the peak cortisol level, we should consider about the lower cortisol binding protein in hypoalbuminemia group. So the level of cortisol should be carefully interpreted in patient who was hypoalbuminemia.

There are 2 studies about the basal cortisol levels and the response to dynamic test for adrenal insufficiency. The study by Hagg E, et al. in 1987 showed that basal cortisol of >10.875 mcg/dl almost excluded and of <3.625 mcg/dl strongly suggest adrenal insufficiency by IIH.[13] And the study from Taiwan in 2002 by Lee MT, et al. studied the relationship between basal cortisol level and the standard dose (250 microgram) ACTH stimulation test in the evaluation of adrenal insufficiency. This retrospective study conducted in 106 unstressed patients with suspected hypothalamic-pituitary-adrenal disease. A mean basal morning serum cortisol of ≥ 10.88 mcg/dl excluded the possibility of adrenal insufficiency and a level of < 3.99 mcg/dl suggested adrenal insufficiency. A mean basal cortisol level of ≥ 8.48 mcg/dl predicted a normal cortisol response in the ACTH stimulation test with sensitivity 80.6% and specificity 91.4%.[21] The clinical or laboratory which can be found in adrenal insufficiency were not shown these studies so we could not compare about the clinical or laboratory clues with our study.

The limitation of our study was the retrospective study which some data were incomplete. The majority of cases in our study were secondary adrenal insufficiency from exogenous steroid usage which may not apply to every causes of adrenal insufficiency. Even though the ACTH stimulation test is not the gold standard for diagnosis of adrenal insufficiency, our study is the real practice which the insulin induce hypoglycemia is not frequently used. The major causes of our investigation about adrenal insufficiency were history of steroid usage (20.3%), unknown cause of shock or hypotension (19.2%) and hyponatremia (18.6%) which were in real life. The mainly results of the ACTH stimulation test in this study were not diagnosed adrenal insufficiency (63.3%) which showed the unnecessary test. The knowledge from this study could help us to make the suitable decision to perform ACTH stimulation test by the basal cortisol level and the clinical e.g. the history of steroid usage, the cushinoid appearance and eosinophilia.

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